UNIVERSITY OF GHANA

HUMAN HEALTH RISK ASSOCIATED WITH POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) CONTAMINATION OF REPEATED USED EDIBLE OILS AND COMMONLY CONSUMED FINGER FOODS IN GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN NUCLEAR AND ENVIRONMENTAL PROTECTION DEGREE

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DECLARATION

This declaration is the result of research work undertaken by Issaka Sam Suraj in the University of Ghana, under the supervision of

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DEDICATION

This thesis is dedicated to my late parents Mr. and Mrs. Issaka Abdallah Yabara for taking care of me during my early school days. Am grateful for the opportunity given me and I pray for God's mercy upon their souls even as they rest in the bosom of Allah.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
GC	Gas Chromatography
MS	Mass Spectrometry
PAH	Polycyclic Aromatic Hydrocarbons
HI	Hazard Index
HM	High Molecular
LP	Lower Molecular
MM	Medium Molecular
JECFA	Joint Expert Commission for Food Additive
MEQBAP	Benzo(a)Pyrene Mutagenic Equivalent
SCF	Scientific Committee for Food
IQ	Intelligence Quotient
DNA	Deoxyribonucleic Acid
IARC	International Agency for Research in cancer
PAH	Polycyclic Aromatic Hydrocarbons
EC	European commission
GLOBACON	Global Telecommunication
POPs	Persistent Organic Pollutant
MMW	Medium molecular weight

HMW	High molecular weight
LMW	Low molecular weight
UV	Ultraviolet
ATSDR	Agency for Toxic Substances and Disease Registry
MDH	
US EPA agency	United State environmental protection
HPLC	High performance liquid chromatography
PLE	Pressurized Liquid Extraction
SFE	Supercritical Fluid Extraction
UAE	Ultrasound Assisted Extraction
MAE	Microwave Assisted Extraction
QuEChERS	Quick,Easy,Cheap,Effective,Rugged, andSafe
FDA	Food and Drug Authority
GSA	Ghana Standard Authority
GL	Guideline
PSA	Primary and Secondary Amines
LOQ	Limit of Quantification
LOD	Limit of Detection
TEF	Toxicity Equivalent Factor
MEF	Mutagenicity Equivalent Factor
MEQBAP Equivalent	Benzo(a)PyreneMutagenic
BW	Body weight
EDI	Estimated dietary intake
MOE	Margin of exposure

ILCR	Incremental Lifetime Cancer Risk
EF	Exposure Frequecy
ED	Exposure Duration
CF	Conversion Factor

ABSTRACT

The methods used in food preparation, be it boiling, frying, baking or roasting may have a significant effect on the food. In Ghana, frying and baking are among the common methods used in the preparation of finger foods. In the case of deep frying, the repeated use of same oil for frying different batches of foods is a cooking habit among most Ghanaians. This study looked at how repetitive frying sessions affect the compositions and concentrations of polycyclic aromatic hydrocarbons (PAHs) in three commonly used edible oils with bean cakes, doughnuts, and plantain chips. Possible human health risk associated with the consumption of contaminated edible oil and the selected finger foods was estimated. The PAH levels in the oils were measured before and after each of the three consecutive frying cycles. Beans cake, doughnuts, and plantain chips were fried with each oil type in session and the PAH content was measured. The PAHs were identified and quantified using GC/MS. Consumption of these oils and finger foods has been linked to both carcinogenic and non-carcinogenic human health risks. This study's findings revealed the presence of 15 of the 16 target PAHs in all samples at varying concentrations. The three unused oils recorded seven PAHs (Nap, Ad, flr, Ant, Flu, Pyr, and B(b)F) primarily in the low ring (2-4 rings) out of the sixteen PAHs investigated. After multiple batches of fry, the sum of 4PAH levels were 588 µg/kg, 352 µg/kg, and 752 µg/kg for sunflower oil, mixed vegetable oil, and soya bean oil respectively. These levels were significantly higher than the 10 µg/Kg limit set for edible fats and oils by European Union Regulation number 836/2011. Benzo[a] Pyrene concentrations of 13 µg/kg and 24 µg/kg were found in the second and third fry of beans cake in soya bean oil, respectively. In addition, the third fry of mixed vegetable oil with beans cake and plantain chips recorded 3 µg/Kg and 20 µg/Kg, respectively. These

figures exceeded the European Commission's limit of 2µg/kg for fats and oils in food. The concentrations and compositions of PAHs were also discovered to vary based on the oil type, finger food, and frying sessions. Increase in percentage for HM-PAH in the second and third fries were 22% and 40% higher, respectively, than in the unused oil sample. After three frying sessions, the hazard index (HI) values as non-cancer indicators ranged from 0 to 4.45, and the lifetime cancer risk values for the used vegetable oils (2.24×10^{-5} to 2.17×10^{-2}). The cancer risk values for finger foods ranged from 1.83×10^{-5} to 6.88×10^{-5}. The no-cancer and cancer risk values for the used oil and finger foods were both high, indicating significant health risks linked to the intake of these edible oils and foods, particularly among children.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Consumption of contaminated food has been identified as a significant source of exposure to toxic substances that may be hazardous to human health. Exposure to potentially hazardous substances in food has been linked to severe health consequences. Although the presence of trace levels of hazardous substances in food is considered a sign of contamination, the risk of adverse health effects is dependent on levels, incidence of encounter, and length of exposure. Foods processing can contaminate food with potentially toxic compounds. PAHs may be introduced into the food from the food processing techniques. However, the type and source of materials used to prepare the food have the potential of influencing the level of contaminants in the food.

Finger foods commonly consumed in Ghana are made from cereals, cassava, plantain, yam, potato etc. The processing of cereal based foods and others involve drying, smoking, roasting at high temperatures, grilling and milling. These processing routes can introduce PAHs in the food (Essumang et al., 2012).

Contamination of commonly consumed finger foods exposes people to toxic substances such as PAHs, which can have negative health consequences. Ingestion of PAHs in food has been linked to a variety of health problems that are a major cause of morbidity and mortality. PAHs and their compounds have been identified as potentially genotoxic and carcinogenic to humans, making them a priority group in assessing the risk of long-term

adverse health effects (JECFA, 2005; SCF, 2002). Food safety is an increasing global worry, and if PAHs are found in food at levels above recommended levels, this could present public health concerns. Low IQ damaged DNA in unborn babies, Growth retardation, low birth weight, disruption of endocrine systems, Increased risk of cancer and reproductive are among the health concerns of exposure to PAHs (Anyakora et al. 2004; Kim et al, 2014, IARC report 2010). Protracted or severe exposure to PAHs has been associated with reduced immune system function, vision loss, damaged kidneys and livers (e.g. jaundice), respiratory related difficulties, irritation, fertility issues, lung, skin, intestinal system, bladder, scrotal cancer, neurodevelopmental effects, and neurotoxicity in laboratory animals (Digg et al., 2013). The health effects of PAHs are largely determined by the length and manner of exposure, the amount of PAHs exposed, and the relative potency of the PAHs (IARC report, 2010). Subjective factors such as pre-existing medical status, age, and the manner in which the exposure occurred can also have an impact on health. High temperatures were used in the roasting, frying, and grilling of food, which has been reported to account for largest causes of PAH in the food (Srogi et al, 2007, Chang et al, 2006), as a result of the increase in temperature, two ways have been observed: first, heated oil evaporate into the air, and second, the PAHs are produced by pyrolysis from partly broken carbon-based molecules in food and edible oils. As a result, several studies on the levels of PAHs in edible vegetable oils and other food matrices have been conducted.

Many countries, including Brazil (Rojo et al. 2011), Korea (Kang et al. 2014), China (Zhao et al. 2018), Iran (Yousefi et al. 2018), and Egypt, have found high PAH concentrations in vegetable oils (Jin-Ku et al.2021). Nonetheless, little information about the PAH content of raw or fried oil is available in Ghana. PAHs are typically formed in edible oil during

manufacturing techniques such as direct fire drying, in which combustion products may come into contact with the oil seeds or oil (Speer et al., 1990; Standing Committee on Foodstuffs, 2002). PAH emissions from cooking are also heavily influenced by food fat content, amount of food cooked, and cooking method. Food with a higher fat content emits more PAHs than food with a lower fat content (Zhu et al, 2007). A comparison of cooking methods revealed that boiling produced the fewest PAHs, whereas broiling and frying generate the most (Chang et al, 2006).

Plant oils are widely promoted as superior to animal fats in Ghana due to their relatively high unsaturation content. However, the manner in which these previously good oils were sold makes them more susceptible to quality defects. Poor quality edible oils contain trans fats as a result of hydrogenation and thus pose a number of health risks due to high levels of free radicals. Free radicals promote tumor growth, raise the risk of coronary artery disease, inflame blood vessels, and inhibit key enzymes that regulate blood flow. Trans fats have been linked to prostate cancer, colon cancer, and breast cancer (Jin-Ku et al., 2021). In Ghana, there are several types of vegetable oil Some imported ones are brought into the country in large unpacked quantities in barrels, which are then packaged into gallons and sold to consumers. Edible oils of various types are used in the preparation of various dishes using various cooking methods. Frying is a popular way to prepare foods like plantain chips and fried yams. In the preparation of such foods, edible oil is used several times to fry the foods at extremely high temperatures.

1.2 PROBLEM STATEMENT

In Ghana, tonnes of edible cooking oils both locally produced and imported are consumed. Edible cooking oil is used to prepare a variety of dishes in Ghana, most of the oils are repeatedly used by food vendors hence, and its contamination may lead to serious public health issues. PAH contamination in cooking oil has been reported in studies by various authors. Both the Scientific Committee on Food and the Joint FAO/WHO Expert Committee on Food Additives have identified nine PAHs as genotoxic and carcinogens (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[b]fluorene, benz[a]anthracene, benzo[k]fluoranthene, and benzo[a]pyrene) (SCF, 2002, JECFA,

2005). Eight PAH congeners are currently classified as known carcinogens in Annex VI of Regulation (EC) 1272/2008. (CLP regulation). According to Annex XVII of Regulation (EC) 1907/2006 (REACH regulation), substances or mixtures containing these eight PAHs in concentrations above certain limits must be classified/labeled as carcinogenic and may not be sold to the general public. Cancer is one of the world's leading causes of death, accounting for nearly 10 million deaths in 2020 (WHO, 2021). In Ghana, national figures on cancer keep increasing every day but information about causes are scanty. According to 2021 GLOBACON report, twenty-four thousand and nine (24,009) people were diagnosed with different types of cancers in Ghana for the year 2020. Food contaminated with PAHs has been linked to cancer, but information on the contamination and quality of cooking oil samples, as well as repeatedly used cooking oil samples, is lacking. Consumption of contaminated foods without knowing their safety may result in a public health crisis. As a result, a comprehensive assessment of PAH levels in fresh oil, used cooking oils, and some fried foods in Ghana is required

1.3 MAIN OBJECTIVE

To evaluate the compositions, concentrations, and sources of PAHs in fresh and used oil, as well as commonly consumed finger foods in Ghana, in order to determine the potential human health risk associated with consumption**.**

1.4 SPECIFIC OBJECTIVES

The specific objectives of the study are to:

- Assess the distribution and concentration of PAHs in edible oil and selected finger foods.
- Characterize changes in PAHs in Edible Oils during Repeated Frying Process.
- Estimate possible human health risks of consuming such foods and oils

1.5 RELEVANCE AND JUSTIFICATION

Convectional food processing techniques have the possibility of being a significant source of PAH contamination in foods**.** Grilling, frying, baking, smoking and drying are all known causes of PAH contamination in food (Essumang et al., 2014 and Rozentale et al., 2017). Repeated frying of the same oil may result in the accumulation of PAHs in both the oil and the food.

In Ghana, many people patronize fried foods (plantain chips, doughnut, beans cake etc) on daily basis either as snack or main meal combined with other foods. Vendors of such foods more often used the oil several times to fry such foods and hence may accumulating PAHs in both the foods and the oil. Due to the nature of how such foods are prepared especially by food vendors, PAHs contamination may results and this have dire health issues to the consumers. Consequence to that, it is critical to assess PAH levels in fresh as well as used

cooking oil, as well as some food items fried with such oils, and to evaluate the risk linked with their consumption.

CHAPTER TWO

LITERATURE REVIEW

2.1 BACKGROUND

PAHs are Persistent Organic Pollutants (POPs) found in compounds that contain carbon. They contain two or more benzene rings and they burn in the air to form particles. PAHs have been found in high concentrations in environmental matrixes as well as food, and are thus thought to be ubiquitous in the environment. PAHs are ubiquitous because they are formed and released in all organic material processes. During the industrial revolution, significant increases in PAH concentrations in the natural environment were first reported in 1988.

2.2 SOURCES OF PAHS

Three distinct processes are used to extract PAHs from compounds containing carbons Latimer, 2003. The first process is pyrogenic, which involves hightemperature organic pyrolysis (350°C - 1200°C). The petrogenic process, which involves the formation of coal and oil from organic matter at average temperatures (100°C - 150°C), is the second source. A few examples include oceanic and oil spills caused by tank leaks. The third process is biological, and it includes biosynthesis by microbes and plants, as well as vegetative matter decomposition.

The conscientiousness, on the other hand, is centered on man - made sources of PAHs, which can be domestic, industrial and agricultural. Numerous environmental activities contribute to the

formation of PAHs in the environment. Figure 2. 1 depicts the contribution of PAHs to the environment.

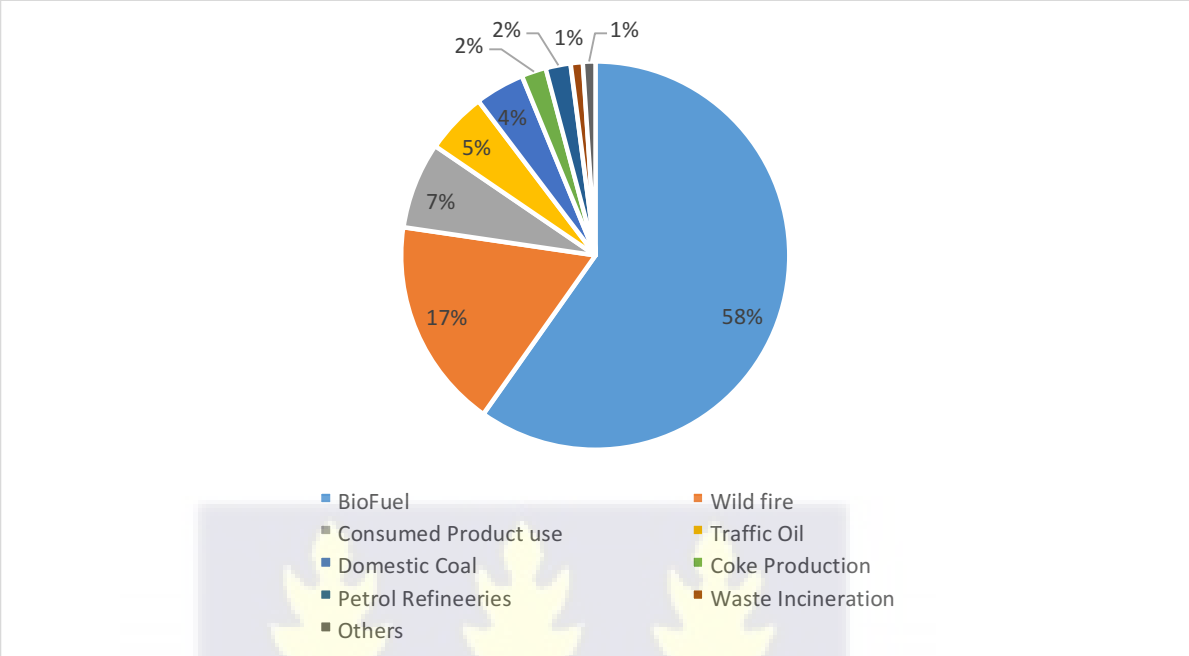

Figure 2. 1 Environmental sources of PAHs. (Adopted from Viviana Galie theses, 2019)

2.3 CHARACTERISTICS OF PAHS

PAHs are solids organic compounds with low vapor pressure, high boiling point, high melting and extremely low aqueous solubility. As molecular weight increases, the latter two parameters tend to decrease, whereas resistance redox reaction increases. With each additional ring, the aqueous solubility of PAH decreases. This means the most water soluble PAH is naphthalene. For the purpose of classifications Naphthalene to Pyrene are known as light PAH and the rest with higher water solubility water are classified as the heavy PAHs. Meanwhile, due to their high lipophilicity, PAHs dissolve faster in organic solvent. Each PAH congener has a distinct UV absorbance spectrum which is helpful in

detecting PAHs. When most PAHs are excited, they emit specific light wavelengths (when the molecules absorb light) which is useful for their detections in compounds.

2.4 FORMATION MECHANISM OF PAHS

Formation of polycyclic aromatic hydrocarbons is thought to follow two steps. The first step is the partially breakdown of carbon base molecule into smaller unsteady units at high temperatures (Pyrolysis or pyrolytic cracking). The second step is pyrosynthesis, where the fragments resulted from earlier reaction which are mainly radicals react with each other to form larger molecules which are relatively stable PAHs. The repolymerization process occurs mainly in low-oxygen environments. As the oxygen / fuel level drops, the rate of forming of PAHs goes up; the fragments loose some hydrogen atom, which forms water upon being merged with oxygen during the different phases of the reaction: the carbon-rich fragments mix to produce polycyclic aromatic hydrocarbons, which are more stable molecules with a high C / H ratio. After the cracking and partial combustion processes, there is a frequency of the presence of radical remains containing two carbon atoms that can react with an acetylene molecule to give a radical containing four carbon atoms. (Fig.2.2)

Figure 2. 2 Formation of a chain of C4 radicals

The radicals that are formed recombine with another similar acetylene radicals molecule which cyclized to form benzene ring (Fig 2.3)

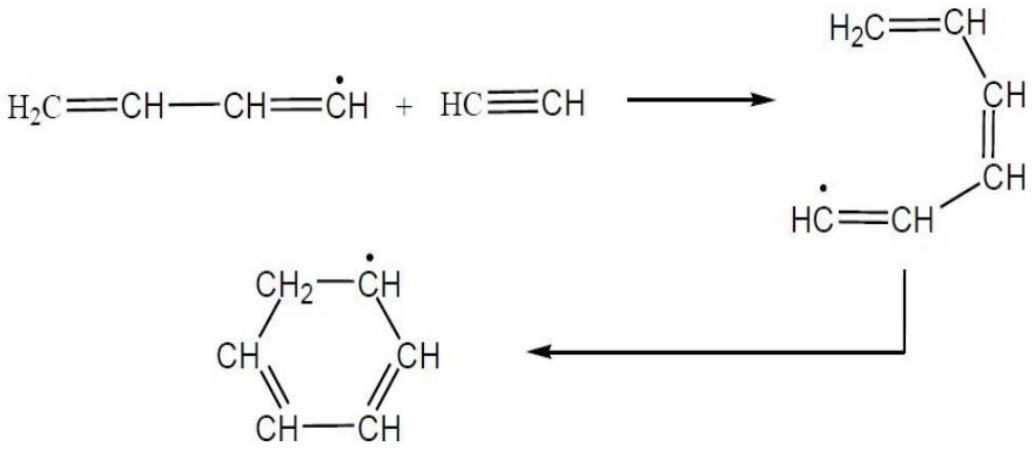

Figure s2. 3 Formation of a 6 terms ring

The reactions continuous and more benzene rings are formed resulting in side chains which formed condensed benzene ring (Figure 2.4)

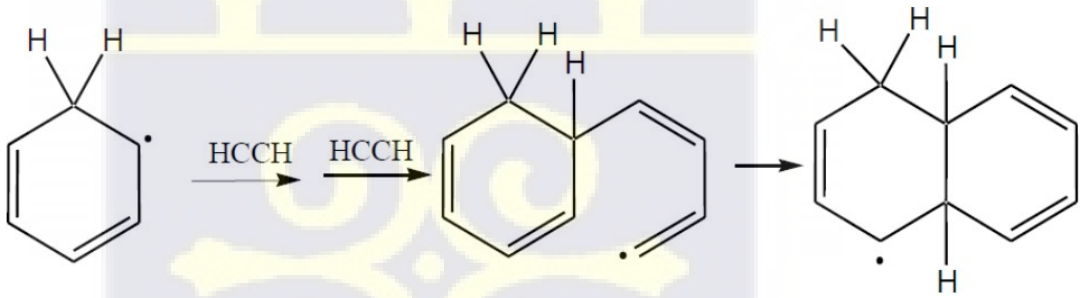

Figure 2. 4 Formation of condensed benzene rings.

Furthermore, some kinds cooking techniques can result in the production of PAHs, particularly heat treatments associated with both product preservation (smoking) and food preparation (grilling, cooking, frying). PAHs in foods can be derived from burning fuel (exogenous formation), typically from incomplete combustion of the fuel, or from the

surface of food (endogenous formation) as a result of harsh heat treatments (high temperatures, long treatment times and proximity to heat sources.

2. 5 TYPES OF PAHS

According to Hao et al., 2016, PAHs are divided into three categories depending on their molecular weight range. PAHs with the same molecular weight behave similarly in the environment. Low Molecular Weight PAHs (LMW) have two or three rings and have molecular mass from 152 to l78 g/mol. The four-ringed medium molecular weight (MMW) PAHs have a molecular weight of 202 g/mol. Fluoranthene and pyrene are examples of this class. The third molecular weight class of PAHs is high Molecular Weight PAHs (HMW). Such as benzo(a)pyrene, dibenz(a,h)anthracene, and indeno(1, 2, 3-c, d)pyrene, they have five to seven rings and a weight of 228 to 278 g/mol (ATSDR, 1995).

The number of rings determines toxicity; PAHs with more rings are more toxic.

2.6 SOURCES OF HUMAN EXPOSURE

The public's exposure to PAHs is a source of constant concern. This problem stems from the fact that PAHs are common environmental pollutants with genotoxicity, embryotoxicity, and carcinogenic properties. Because of this concern, researchers have devised methods for evaluating the general population's exposure to PAHs, one of which is the identification and quantification of PAH urinary metabolites. It should be mentioned that almost all individuals are subjected to PAH mixtures instead of single PAHs. As shown in Table 3, the IARC has classified a number of individual PAHs compounds as probable human carcinogens (Category 2A) or potentially human carcinogens (Category 2B), as

well as evidence of human carcinogenicity (Category 1). Category 3 shows agent or the congener is unclassifiable in turns carcinogenicity (Table 2.1)

Table 2. 1 IARC classification of PAHs into categories based on their carcinogenicity

PAH	MW	No. of aromatic rings	IARC
Naphthalene	128	2/3	2B
Fluorene	166	2/3	3
Phenanthrene	178	2/3	3
Anthracene	178	4	3
Fluoranthene	202	4	3
Pyrene	202	4	3
Benzo(a)Flouren	216	4	3
Benzo(a)Anthracene	228	4	2A
Chrysene	228	4	3
Benzo(b)Fluoranthene	252	5	2B
Benzo(k)Fluoranthene	252	5	2B
Benzo(j)Fluoranthene	252	5	2B
Benzo(e)Pyrene	252	5	3
Benzo(a)Pyrene	252	5	1
Perylene	276	6	3
Benzo(ghi)Perylene	276	6	3
Indeno(1,2,3,cd)Pyrene	278	6	2B
Benzo(b)Crysene	278	6	3
Dibenzo (aj)Anthracene	278	6	-
Dibenzo(ah)Anthracene	278	6	2A
Dibenzo(ac)Anthracen	278	6	-

The chemical structures of some PAHs are shown in Figure 2.5.

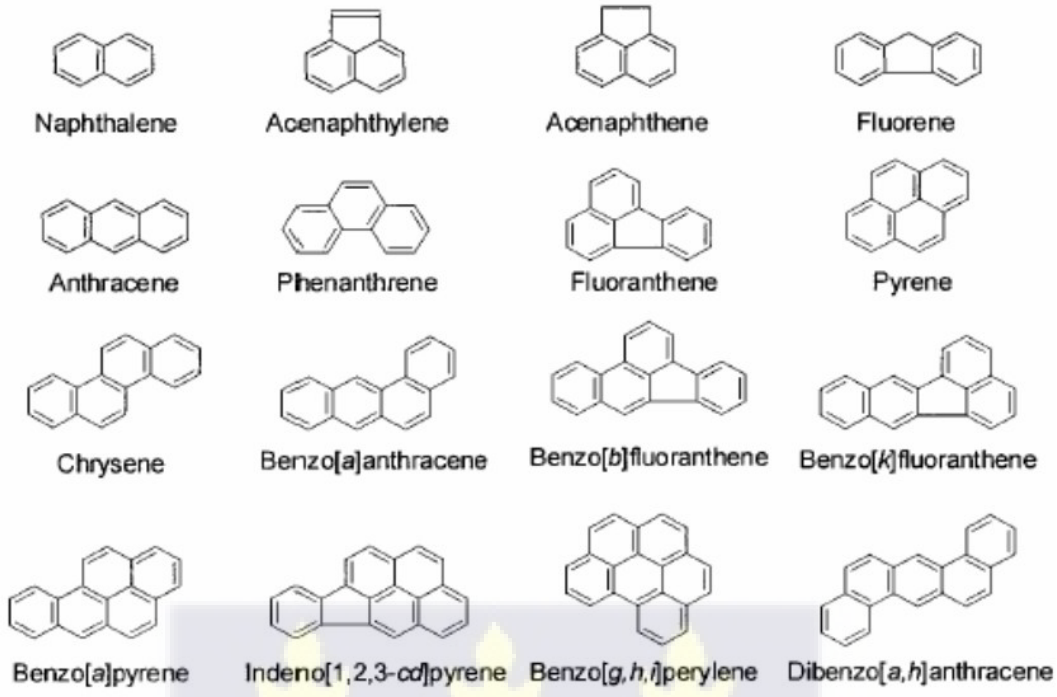

Figure 2. 5 Chemical Structure of Some PAHs

2.7 TOXICITY AND HEALTH EFFECT OF PAHS ON HUMANS

A significant number of PAHs were recognized as carcinogenic to humans in the IARC report (2010). The majority of people are regularly exposed to PAHs. Babies are also susceptible to PAHs contamination. Asomaoh et al., 2019 discovered high concentrations of PAHs in the breast milk of nursing mothers leaving at e- waste recycling areas and residential areas. The route and duration of exposure, as well as the concentration of PAHs, are expected to influence the severity of their effects on human health**.** A person's age and pre-existing health conditions also determine the magnitude of the impact of PAHs on humans. Several reports have mentioned many health issues related to the effects of PAHs on humans. These include, but are not limited to, irritation, decreased fertility, lung, skin,

gastrointestinal tract, bladder, scrotal cancer, developmental neurological effects, and renal toxicity. Meanwhile, repetitive contact with skin may cause inflammation and redness Boström et al., 2002. PAHs are generally thought to be more potent when exposed early in life IARC report (2010).

2.9 BIOCHEMISTRY AND METABOLISM OF PAHS

British Scientist Sir Potter Percival discovered the potential of PAH mixtures to cause human cancer when he isolated B(a)P from soot and rubbed it on the skin of mice. After some he discovered tumors on the mouse's skin, which he robbed of the soot. After further scientific research and theoretical studies, he found that there is a high electronic density region on the B(a)P known as the K region, which is believed to be involved in the carcinogenic activities. Further studies also discovered another region called the Bay region. According to the study, PAHs with both the k and bay regions are potent carcinogens, and those with fjord regions or a sterically hindered bay region are even more potent carcinogens (Figure2. 6).

Brookes et., al, 1964 demonstrating a strong association between carcinogenic PAH interactions with DNA and their carcinogenic potency in the study involving mouse. Based on these findings, Baird and Brookes proposed in 1973 that PAHs were activated in the K-region of the epoxides that bind DNA (Baird et al., 1973). In 1977, Lehr and Jerina (1977) described the theoretical basis of PAH-diol and epoxide carcinogenic activity.

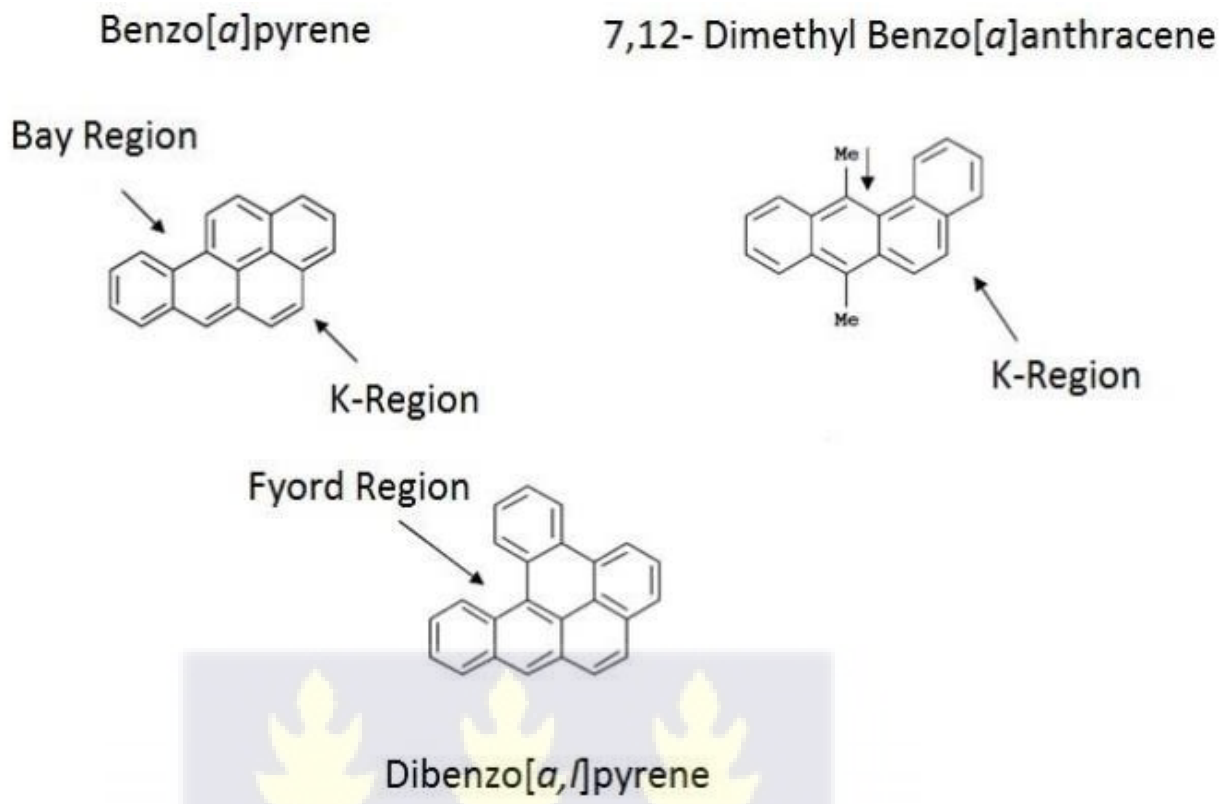

Figure 2. 6 Structural formulae of some PAHs with high carcinogenic Power

The mutational and cancer-causing properties of PAHs are caused by the changes which those compounds undergo during body's metabolic reactions. Carcinogens are byproducts of the metabolism of PAHs, which the body produces to aid in their elimination; in fact, the PAHs are converted by the body into water-soluble rendering derivatives, allowing them to be eliminated more easily (Baird et al., 2005). The following observations led to the decision to use B[a]P as an indicator: the significant correlation, according to the EFSA 2008 report, B[a]P is the most frequently encountered congener in terms of toxic effect and is found in a variety of matrices, both food and the environment. It has been used as a PAH class pointer in terms of contamination and cancer causing ability. This is due to the fact that the studies are epidemiological on a single

component, whereas in nature, the PAHs are always present in mixtures and with precise concentration ratios, depending on the polluting source.

The first step in the transition of benzo[a]pyrene is epoxidation, that is catalyzed by cytochrome P450 in the most reactive positions 7 and 8, known as the K region (figure2. 7)

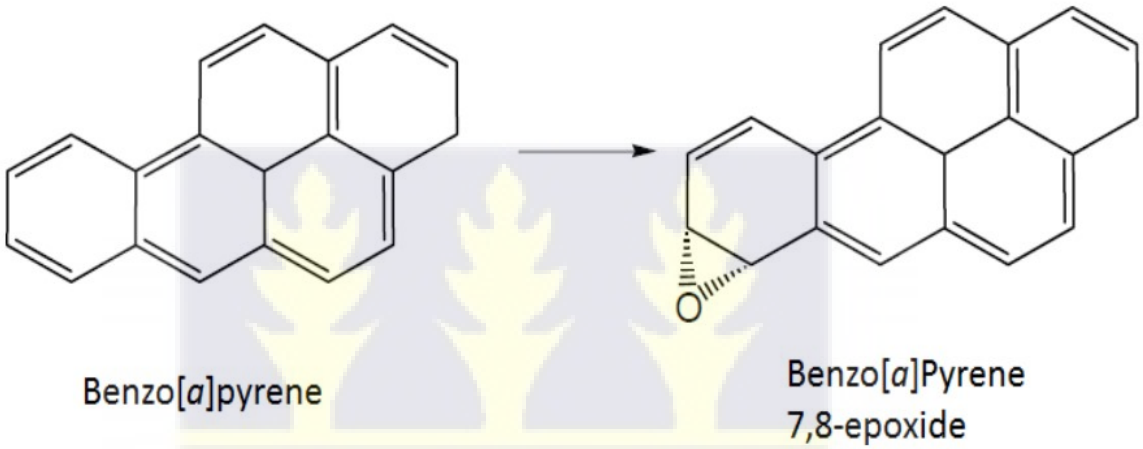

Figure 2. 7 Epoxidation in positions 7 and 8.

Water attacks the epoxide resulting in the formation of a diol, which is more water-soluble and thus more easily removed (Figure 2. 8).

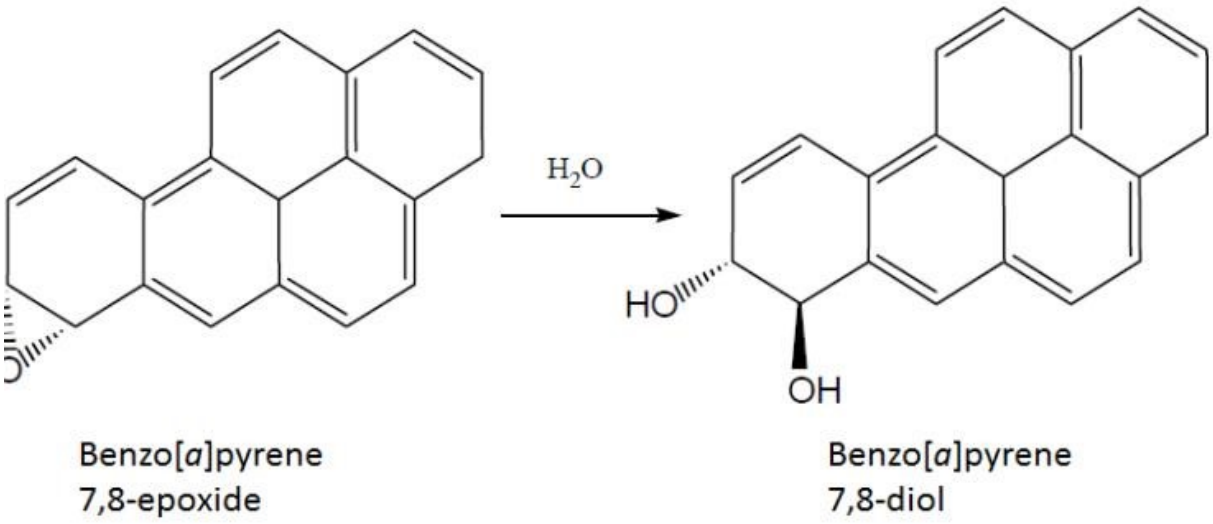

Figure 2. 8 Formation of a diol

Cytochrome P450 could then reduce this PAH metabolite to form an epoxide diol (Figure 2.9).

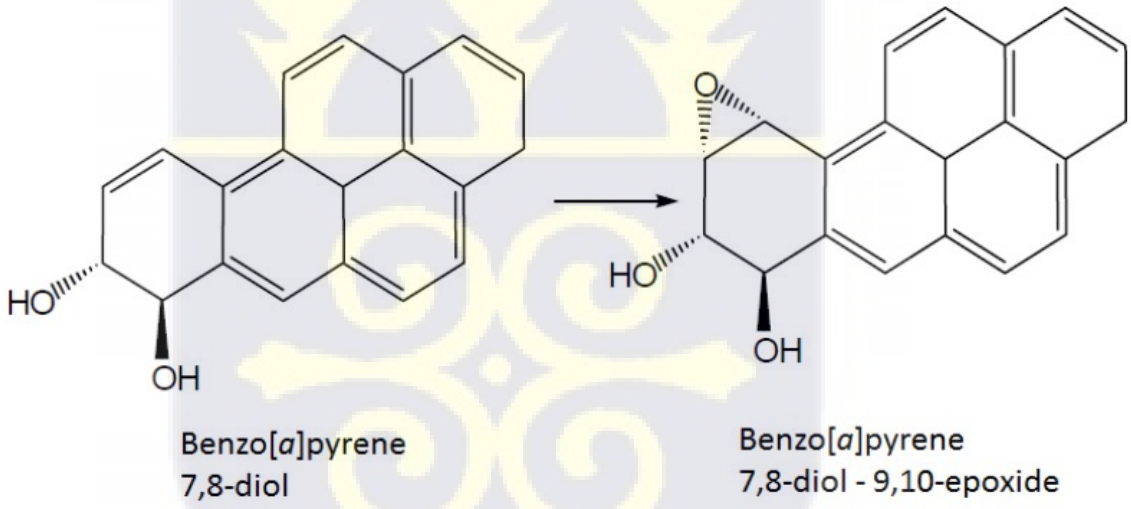

Figure 2. 9 Metabolism of Benzo[a]pyrene

This compound, like adenine, is considered as the actual carcinogenic species that attaches to DNA via nucleophilic attack (Figure 2.10). The large hydrocarbon residue's covalent

attack causes obvious DNA damage, resulting in mutations and an increased likelihood of carcinogenicity.

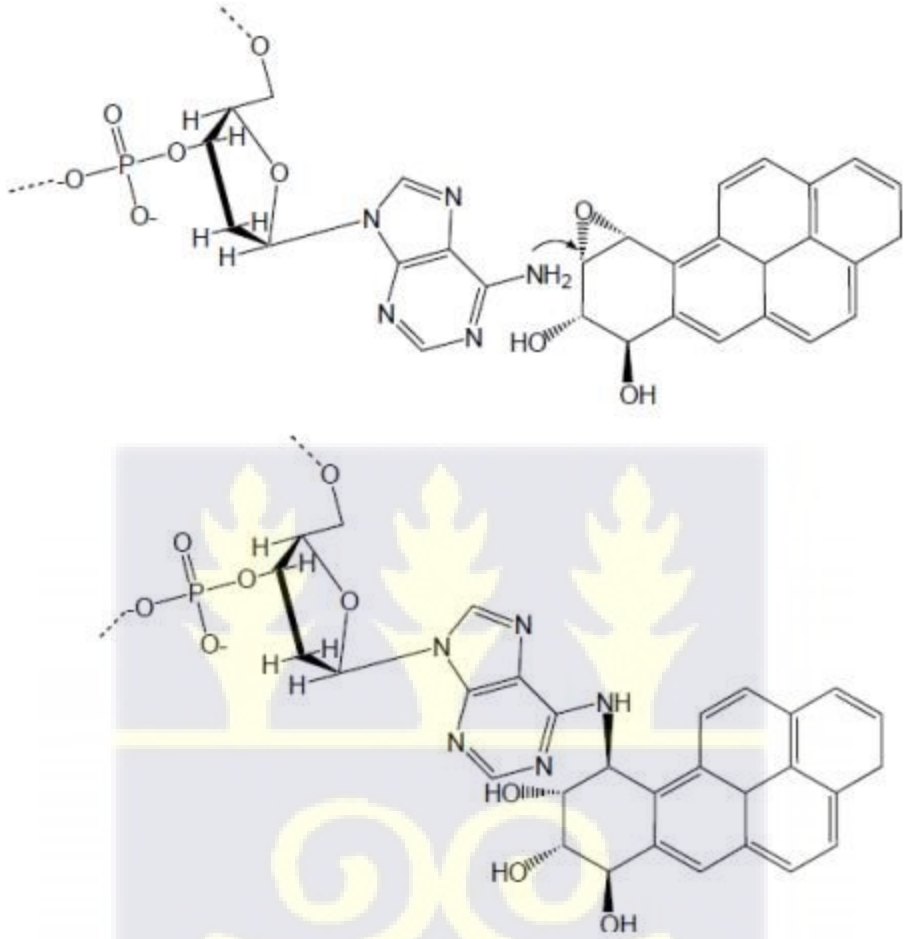

Figure 2. 10 Reaction of Benzo[a]pyrene 7,8 diol-9,10 epoxide with DNA

The understanding of the mechanism of PAH cancer development, where the induction of genetic changes plays a causal role, allows the experiment's observations on animals to be extended to humans while excluding indirect, species-specific mechanisms. However, additional research into the carcinogenic effects of individual PAHs on humans has been conducted. In reality, human exposure involves complex combinations of PAHs that frequently contain other carcinogenic

components. Based on laboratory toxicity testing, benzo[a]pyrene is labeled as a "initiator," or a congener that, after metabolic action, reacts with DNA, causing negative consequences that are also present during cellular division and thus transferred to offspring. As a result, benzo[a]pyrene is classified as genotoxic and capable of causing mutations (Nesnow et al., 1998).

2.10 OCCURRENCE OF PAHS IN FOOD

Polycyclic aromatic hydrocarbons Contamination of food ingredients occurs in different forms. Industrial activities involving heating, drying and smoking, which liberate combustion products, can be significant contributing sources. PAH contamination of vegetable oils is primarily caused by some of the above-mentioned technological processes, in which byproducts come into direct contact with grain, oilseeds, or oil. These home cooking methods produce PAHs. PAH levels in grilled and charcoal-grilled foods (including fatty meats and meat products grilled under long and severe conditions), traditionally smoked foods (particularly fish) are found to be high. When consumed in large quantities, smoked and grilled foods can significantly increase PAH intake (Bocca et al., 2003). Otoo et al., 2022 have also detected quantities of PAHs in smoked guinea fowl which is one of the delicacies of some Ghanaian.

2.10.1 COOKING OIL

Fats and oils (lipids), which are sources of food, are very susceptible to contamination by polycyclic aromatic hydrocarbons due to the fact that PAHs are lipophilic**.** Triacylglycerols and lipids are typically mixed in varying proportions to form lipids. While natural lipids and oils contain a wide range of fatty acids, only a few are required in the diet of humans. Edible oils, which account for a

significant amount of the total daily calorie intake, are an essential component of a properly balanced and healthy diet. According to FAO/WHO (2010) recommendations, fats should account for 20% to 35% of total daily energy intake. Edible oils contain fatty acids, for example linoleic, oleic etc which the body cannot produce and must be obtained from food. The chemical and physical qualities of fats or oils that are needed for any specific reason as stated in a product description or certificate of analysis are referred to as oil quality (Matola et al., 2015) Input materials growth cycle, soil fertility, raw material post-harvest storage conditions, drying process of oil seeds prior to oil extraction, by direct contact with combustion gases, and post-process storage conditions such as heat and exposure to air are some of the factors that impact the quality of cooking oils, according to (Turner R., 2010).

Oil oxidation is a key contributor to quality loss, and several authors have previously identified the primary causes of oxidation in edible oils as processing procedures, temperature, light, and oxygen. Lipid oxidation, according to Mehmood et al. (2012), has a negative impact on various aspects of the oil, including taste, aroma, and nutrition. It also induces biological damage of living tissues and increases the risk for cardiovascular disease, in addition to other health issues such as diarrhoea and slow growth rate.

Because of their relatively high levels of unsaturation, plant oils are widely promoted in Ghana as superior to animal fats. However, the circumstances in which these previously good oils are prepared and sold make them more susceptible to quality defects. Poor quality vegetable oils are high in trans fats as a result of hydrogenation, posing a range of health threats given the high levels of free – radical. Free radicals encourage tumor development, raise the likelihood of coronary artery disease, inflame blood vessels, and inhibit key

enzymes that regulate blood flow (Trinity et al., 2016) Trans fats have been linked to prostate cancer, colon cancer, and breast cancer.

2.10.2 Influence of Refining of oil on PAHs Content in Edible Oil

Although the amount of PAH contamination in unrefined cooking oils varies, refined edible oils have low levels than unrefined oils, which could be attributed, at least in part, to the reduction observed during refining. According to Anjom-Shoae et al., 2019, when the authors assessed the impact of various refining steps such as deodorization, bleaching and neutralizations on crude soybean and sunflower oil PAH content, they discovered a significant reduction in PAH content, particularly light PAH. Total PAH decreased by 72% in sunflower oil and 87% in soybean oil after refining. Deodorization even though was observed to have little effect on heavy PAHs, does seem to have the biggest effect on lowering total PAH levels among various steps in the refining process, which is in line with previous studies. Whereas activated charcoal treatment mainly reduces higher condensed heavy PAH. It seems that the type of treatment employed in the bleaching step is essential. Some works showed a rise in light PAH content after bleaching, probably due to the use of contaminated clay. When compared to activated earth or clay, activated charcoal can reduce heavy PAH content more effectively

2.11 EFFECT OF COOKING METHOD ON PAHS FORMATION

The proportion of PAH formed during cooking is influenced by the conditions and the technique used. Contact of oil seeds or cereals with combustion gases during drying methods has been found to result in the formation of PAH and therefore must be avoided. Simple practices such as selecting preferentially lean meat and fish, avoiding food contact

with flames when barbecuing, using less fat when grilling, and cooking at a lower temperature for a longer period of time lead to lower PAH contamination of foods (Lijinsky and Ross, 1967). When fat is dripped onto an open flame, a column of smoke rises and coats the food with PAH. Using medium to low heat and moving the meat away from the heat source can greatly reduce PAH formation. The depth of brown color of grilled foods does not always correspond to the intensity of flavor. It is not necessary to overcook the food to obtain flavor. In contrast, cooking should always be efficient in terms of inactivating any possibly contaminating bacteria or endogenous toxins.

2.12 CARCINOGENICITY

PAHs enter the human body through three different pathways. Inhalation, dermal absorption, and ingestion are all examples of this. The byproducts of PAH metabolism can be toxic to human body cells. The DNA and cellular proteins were found to bind with PAH metabolites such as epoxides and dihyrodiols (Armstrong et al., 2004). These reactions may result in tumors, teratogenesis, and mutation, all of which can lead to cancer and cell damage. Data show that PAH mixtures are extremely toxic to cells and can cause cancer. This evidence was derived from data collected from PAH-exposed workers in the workplace. Skin, gastrointestinal, and lung cancers were among the health problems observed in this group. Even so, because workers were also exposed to other cancer causing agents (e.g., aromatic amines), it is uncertain whether exposure to PAHs was the main reason in these research (Bach et al., 2003). In laboratory studies, animals exposed to high levels of some PAHs developed lung cancer from inhalation, stomach cancer from ingesting PAHs in food, and skin cancer from skin contact (US EPA Factsheet, 2008).

Benzo(a)pyrene is noteworthy because it was the first chemical carcinogen discovered. This is also the most common PAH in animals that causes cancer. A number of PAHs are carcinogenic to animals, according to available evidence (US EPA Factsheet, 2008), and some PAH-rich mixtures are also carcinogenic to humans according to IARC report (2010).

2.13 ANALYTICAL TECHNIQUES OF PAHS

Analytical techniques for determining PAHs in food materials vary. Even so, with an increasing number of studies and samples being collected using contemporary confiscated and highly sensitive instruments, some of these designed systems make obtaining correct and timely results extremely difficult. PAHs are extracted from multiple matrices using a combination of two or more solvents with different polarities because they are soluble in a wide range of solvents.

2.13.1 Extraction techniques of PAHs

The extraction technique is chosen for a variety of reasons, including effectiveness, technique prominence, ease of laboratory implementation, cost, and long term projections for advancement through modification. Soxhlet extraction method, though effective require more solvent and ample time foe extraction was but modern techniques are gradually taking its place. It is not a suitable extraction method for a large number of samples due to the long extraction time and use of a large amount of solvent per extraction batch. The modern method, which employs automated extraction techniques, speeds up solvent extraction processes in order to reduce time spent and solvent amount for high recoveries. Among these methods are pressurized liquid extraction (PLE), which is very expensive, supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE), and microwave

assisted extraction (MAE). Another popular extraction method for extracting PAHs from liquid matrices such as edible oil is the Liquid-Liquid Extraction (LLE) procedure. Another quick and easy extraction technique that has proven useful in extraction procedures is QuECHERS extraction

2.13.2 The QuEChERS Extraction Technique

QuEChERS is a short form for Quick, Easy, Cheap, Effective, Rugged, and Safe. This extraction technique has a number of advantages above other traditional methods, such as Soxhlet extraction, which takes a long time and wastes a lot of solvent. Thus, the benefits of using environmentally friendly solvents in the QuEChERS method include faster extraction, simpler steps, lower costs, effectiveness, toughness, low solvent consumption, high recovery rate, and safety (Urkude & Dhurvey, 2015). The QuEChERS method has been successfully applied to PAH analysis (Escarrone et al., 2014; and Urkude & Dhurvey, 2015).

2.13.3 Cleanup methods

Large or related molecules are removed during the cleanup process to protect and ensure the integrity of the analytical system. This assists in ensuring that the analyte of interest and its concentrations are accurately achieved. The liquid-liquid extraction technique is most likely the widely used in the extraction of oil samples. As a result of this extraction process, some ruminant compounds with properties similar to PAHs come along with the extract as co-extracts that must be removed, thus the importance of cleanup excises (El Hawari et al., 2017).

2.14 LEGISLATION

The Stockholm Convention on POPs in 2009 took polycyclic aromatic hydrocarbons into account. Ghana has since amended the convention, and it is now required to take appropriate steps to protect people and the environment from PAHs, or to reduce their release to the greatest extent technically feasible and economically acceptable. Indeed, Article 3(5) of the Stockholm Convention's POPs Protocol requires Convention members to make efforts to reduce PAH release to levels recorded between 1985 and 1995. Because PAHs are a diverse group of chemicals, four pointer compounds for use in emission inventories have been identified: benzyl(a)pyrene, benzyl(k)flouranthene, benzyl(b)flouranthene, and indeno (1.2.3.cd) Pyrene

In Ghana, no adequate actions for handling and managing PAHs have been published, in violation of Stockholm Convention Article 3(5). Act (917) of Ghana Environmental Protections (EPA), which seeks to protect the environment from pollution and hazards, and Electronic Waste Control and Management Act 2016 are relevant provisions in the country for pesticide and waste regulation. The country requires legislation focusing solely on PAHs in order to help regulate the release of such compounds into the environment and protect citizens' health.

2.15 PREVIOUS STUDIES ON PAHS CONTAMINATION

The contamination of Polycyclic aromatic hydrocarbons has reached epidemic proportions, having a negative effect on the human health and the environment. Because PAHs are ubiquitous, contamination of food and environmental matrices is unavoidable. PAHs in foods and edible oils are becoming increasingly studied. Several PAHs have been identified and quantified in foods and edible oils from other countries, and the devastating effects of

PAHs on human health, even at very low concentrations, call for increased concern. Despite the fact that much research has been conducted on food substances, little research on edible oils has been conducted in Ghana. Table. 2.2 summarizes the PAHs range found in edible oils in some countries.

Table 2. 2 Data on PAHs in Edible Oils

Country	Tested Oil	Levels of PAHs Detected	References
Portugal	Sunflower and olive oils	15 PAHs (8.78–26.35 ng/g ww)	Teixeira et al. 2007
Kuwait	Olive,corn, sunflower, sesame, palm olein, soya, canola, mustard, and peanut oil	16 PAHs (34.51 ng/g ww)	Alomirah et al. 2010
Turkey and Spain	Olive Oil	15 PAHs (17.65 and 138.99 ng/g ww)	Ergönül and Sánchez 2013
China(Shandon)	Edible oil	15 PAHs (54.37 ng/g ww)	Jiang et al. 2015
China(Beijing)	Edible oil	16 PAHs (3751.9–7585.8 ng/g	Hao et al. 2016
Iran	Edible oil and fried oil		Yousefi et al. 2018
Egypt	Corn oil, sunflower oil, olive oil, and canola oil	15 PAHs (14.39ng/g -197.42ng/g	Jin-Kui et al 2021

CHAPTER THREE

METHODOLOGY

This chapter describes various activities ranging from market research to the selection of oil brands sold at various marketing centers in the Greater Accra Region, as well as the sampling of cooking oil, sample preparation, and detection of PAHs concentrations in cooking oil and some foods. There are also methods for calculating potential human health risks presented**.**

3.1 MARKET SURVEY

The initial field work involved a market survey where the staffs of Ghana Standard Authority and Food and Drugs Authority where interacted with in order to establish the work done on the PAHs in the cooking oil. The market women and the food vendors were also contacted to ascertain the oil brands that are commonly used for the frying of such foods. Some major marketing centers in the Greater Accra Region which forms the sales outlet for cooking oil to food vendors such as Makola market, Madina market and Nima market were visited. The selected markets for the sampling are the densely populated markets where most food vendors purchase the cooking oil and the markets have different types of cooking oils. The sampling markets mostly operated as open-market system. Hence, there are different cooking oil products both locally produced and the imported brands. A greater proportion of the food vendors in the designated metropolitan area depend on the open-market system for most of their cooking oil supplies.

In this study, cooking oils were obtained from market women who sell common brands used by food vendors for frying plantain chips, beans cake, and doughnuts. The gallons of all three brands bore FDA registration numbers. Following up with the Ghana Foods and Drugs Authority confirmed that the products were indeed registered. The food vendors believe that the cooking oils sold in these areas are healthy, and the prices are reasonable**.**

3.1.1 Selection Criteria for oil and food

The selections of 3 out of several oil types on the market were done on the following considerations:

i. The type of which indicated that the selected oil types were those mostly purchased by the food vendors for the purposes of frying food items and these was confirmed by the market women who sale mainly edible oil at the various markets centers that were surveyed.

ii. The food items selected for purposes of frying represented the most common food items which are fried with the oil on repeated basis. It also represents the common fried food which is consumed by both the young the old as snack or combined with some other meal.

3.2 SAMPLE COLLECTION

Both the cooking oil and the food items were sampled in following protocols set by Greenfield's (2003) for sampling of foods and the Ghana Standard Authority sampling guidelines (GS CAC/GL 50, 2004). Samples were collected at the chosen food vendor's kitchen, where the food is fried. About 25ml of each of the three oil brands was collected into a sampling bottle; the remaining three liters were used to fry three different food items

in turn. Before the food items were fried, samples of the different food items were also taken and the rest were fried. After each round of frying, oil samples and their respective food samples were put into cleaned larger glass containers that were covered and allowed to cool before transferring them into the sample containers; the oil samples were placed in plastic containers, and the food items were wrapped in zipped plastic rubber and placed in a different cooler with ice cubes to maintain the temperature. The duration of the food fry was recorded, as was the temperature of the oil in the pan. Each sample was labeled with a unique identification code before being transported to the laboratory for analysis.

In all, 36 samples were obtained from the three food vendors within the study area, 30 oil samples (fresh 3 and used 27) and 6 food samples (2 plantain chips fresh and fried, 2 beans cake fresh and fried and 2 doughnut fresh and fried) samples.

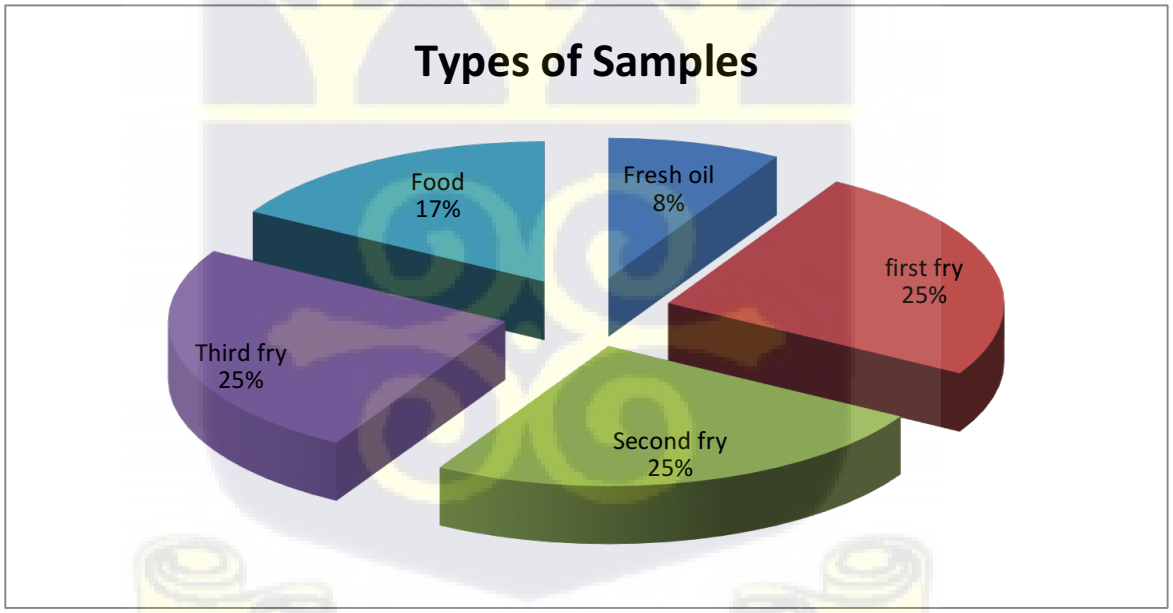

Figure 3. 1 Oil and Food Sample Compositions

3.3 SAMPLE PREPARATION FOR ANALYSIS

The food samples were macerated further to form small particles which can be used for the purposes of extraction and the clean- up processes. The oil samples were left to attain the temperature of the laboratory where the analysis was carried out.

3.4 REAGENTS USED FOR PAHS EXTRACTION AND ANALYSIS

HPLC-grade acetonitrile (99.8%) was used for the extraction. To dissolve the purified extract, pesticide-grade ethyl acetate (99.8%) reagents were used. Anhydrous magnesium sulfate (MgSO4) (99.0%) and extra-pure, analytical-grade sodium chloride (NaCl) (99.0%) were used as extraction salts. Bondesil PSA (Primary Secondary Amines) bulk sorbent 40m was used to purify the extracts. Analytical-grade acetone (99.5%) was used for rinsing glassware. The Ghana Standard Authority Pesticide Residue Laboratory provided the double deionized water used to dissolve and rinse glassware. Benzo(h,h,i)-perylene, benzo(a)-pyrene, benzo(b)-fluoranthene, benzo(a)-anthracene, benzo(k)-fluoranthene, indeno(1,2,3,cd)pyrene, chrysene, anthracene, pyrene, fluoranthene, naphthalene, acenaphylen, acenaphthene, Dibenzo(a,h)-anthracene, Fluorene and Phenanthrene are the sixteen PAHs used in the mixed standards obtained from HPC Standards GmbH, Germany (Appendix) II .

3.5 DETERMINATION OF PAHS CONCENTRATIONS IN THE SAMPLES

The analysis of the PAHs were done an at the Ghana Standard Authority (GSA) Pesticide

Residue Laboratory in Accra. All the 36 samples (both oil and food) were analyzed for the 16 selected PAHs. The oil samples were allowed to stand for some time in order to attain the room temperature and the food samples were stored in refrigerator at 4^{o}C before the analysis.

3.6 SAMPLE EXTRACTION

Two grams of oil were dissolved in 10 mL of 99.8% pure acetonitrile, and the mixture was shaken for a minute, then sonicated for 30 min. At 3500 rpm, the contents were centrifuged. The top layer was then collected in a conical tube. This procedure was carried out twice more. For one and a half hours, the contents of the conical tube were frozen. The solidified fat was then centrifuged for 5 minutes at 3,500 rpm.

For food substances, 5g of the homogenized samples were weighed into tubes. 10ml of distilled water was added and shaken for 30 seconds, add 10 ml of acetonitrile and shake further for 30 seconds. Extraction salts were then added and immediately vortexed for a minute. The content was then centrifuged at 3,600 rpm.

3.7 EXTRACTION CLEAN UP

The clean-up process was performed by measuring 6 ml of the extract in a clean salt containing 900 mg of magnesium sulfate (99.0%), 150 mg of primary and secondary amine, and 150 mg of C18. The contents were shaken and then centrifuged, and 4 mL was transferred into a pear-shaped flask. A rotary evaporator was used to concentrate the content, and 1 mL of 99.8% pure ethyl acetate was used to dissolve it. The extracts were transferred into a 2 mL vial after being sonicated for 20 seconds. GC/MS instrumentation for qualitative and quantitative detection of PAH.

3.8 SAMPLE ANALYSIS

The PAHs were analyzed using a gas chromatography-mass spectrometry system outfitted with an analytical column measuring 30 mm by 0.25 mm fused to a silica-coated capillary measuring 0.25 mm film. At 250 ^{o}C and 2.1 flow velocity, the auto injector was set to a split-less configuration. For one minute, the temperature was set to 80 °C. The temperature was raised to 180 °C at a rate of 25 °C per minute, then to 300 °C at a rate of 5 °C per minute, and held there for one minute. Electron impact (EI) MS was used, as well as a 70electron-volt ionization voltage. The temperature of the ion source was set to 300 °C. With a delay in taking control of the auto solvent, the generated spectra were in the mass range of 45 m/z to 450 m/z. The retention times of the 16 PAHs were quantified and identified using gas chromatography and compared with external standards.

3.9 QUALITY CONTROL AND QUALITY ASSURANCE

The extraction efficiency of the target PAHs was validated using the standard spiked components and recovery methods. The difference in PAH concentrations between spiked and unspiked samples in comparison to spiked samples was calculated and expressed as a percentage (Ciecierska and Obiedzinski, 2010). Table 1 shows the correlation coefficients (R2) for each PAH and regression equation. Analytical calibration curves for dibenz[a,h]anthracene and fluorene revealed linearity coefficients (r2) in the 0.819970.99995 range. (Table 3.1). The average percent recovery was 82–96%, which was within the acceptable range of 70–120%. (CORESTA Guide 5, 2018) Recovery studies in which a blank was spiked with the PAH standard solution yielded precision and accuracy data.

PAH had a detection limit (LOD) of 0.3 µg/kg and a quantification limit (LOQ) of 1 µg/kg. Cross contamination of the samples was avoided by using one pipette per each sample. All weighing instruments were calibrated before use and the glassware were properly washed and rinsed with distilled water and rinsed with acetone and then allowed to dry in desiccator.

Table 3. 1 Linear equation and R^2 of the calibration curves of the 16 PAHs

PAHs	Linear equation	R^2
Naphthalene	y=120.324869 + 401.04	0.99844725
Acenaphthylene	y=111.878394 - 2112.72	0.99966140
Acenaphthene	y = 303.488894+ 6.60	0.99893144
Fluorene	y=535.306544 + 39.72	0.99995164
Anthracene	y=169.953661 - 2493.79	0.99969006
Phenanthrene	y=210.189835 - 4487.89	0.99968539
Fluoranthene	y=103.137622 - 955.82	0.99804055
Pyrene	y=890.336153 - 12994.34	0.99949907
Benzo(a)anthracene	y=2394.729613-27702.55	0.99934970
Chrysene	y=2610.877711 - 139914.03	0.99956648
Benzo[b]fluoranthene	y=3639.331728 - 35865.17	0.99972606
Benzo[k]fluoranthene	y = 3639.298677 - 35094.53	0.99976615
Benzo(a)pyrene	y=2076.498874 - 8260.43	0.99961572
Indeno(1,2,3,c,d)pyrene	y=1515.148344 - 36843.32	0.9997102
Dibenz[a,h]anthracene	y=29.256026 - 456.08	0.81997375
Benzo[g,h,i]perylene	y=2255.282739 - 84558.99	0.99889702

3.10 SOURCES APPORTIONMENT USING DIAGNOSTIC RATIO

Specific PAH compound ratios have been identified as having the potential to distinguish between natural and anthropogenic PAH sources as well as to predict the origin of PAHs, i.e. if the PAHs were released into the atmosphere by petrogenic, pyrogenic, biomass, or coal burning (Tobiszewski, 2014). Two PAHs isomers yields a more accurate estimate of PAH origins rather than using a single PAH due to factors such as volatility and aqueous solubility, partitioning and adsorption, and degradation (Chen et al., 2012). The combinations used in this study to assess the sources of PAHs are shown in table 3.2.

Table 3. 2 Pair of PAH isomers for assessing the sources

PAH Ratio	This Study	Petrogenic	Pyrogenic/Prolytic	Remark
AN/AN+PH	-	<0.5	>0.50	
FL/FL+PYR	-	-	>0.50*	
BAP/BAP+A	-	Up 0.35	>0.35	
IND/INDP+BGHI	-	< 0.2	0.2-0.5	
NAP/NAP+ACL	-	<0.10	>0.10	
Ind/Ind+Dib	-	0.2-0.35	0.2-0.35	Mixed Petro/Pyro

Essumang et al., 2009

3.11 RISK ASSESSMENT OF PAHS

The toxicity, carcinogenicity, and mutagenicity of a large number of PAHs have been well studied and are known to cause serious health problems in humans when exposed to them.

According to the US EPA, seven (7) PAHs (table 3.3) are probable carcinogenic and mutagenic. Benzo(a)pyrene has a toxicity equivalent factor (TEF) of 1.0 and a mutagenic equivalent factor

(MEF) of 1.0. (Durant et al., 1996, 1999; Nsibet and LeGoy, 1992). toxicity equivalent factor (TEF) and mutagenic equivalent factor (MEF) figures for the seven PAHs are shown in Table 3.7 for carcinogenic and mutagenic risk assessments. According to Liao et al., 2005, three age groups were picked for assessing the risk associated with the ingesting of such oils and food samples: child (1-11 years), adolescent (12-17 years), and adult (18-70 years).

Table 3. 3 TEF and MEF of 7 PAHs (Durant et al., 1996, 1999; Nsibet and LeGoy, 1992).

PAHs	TEF values	MEF values
Benzo(a)pyrene	1	1
Dibenzo(a,h)anthracene	1	0.29
Indeno(1,2,3-c,d)pyrene	0.1	0.310
Benzo(a)anthracene	0.1	0.082
Benzo(b)fluoranthene	0.1	0.250
Chrysene	0.001	0.017
Benzo(k)fluoranthene	0.01	0.11

TEQBAP was calculated by using Equation 3.1 (Thi et al. (2016)

$$TEQBAP = \sum (TEFi \cdot Ci) \qquad \text{Eqn (3.1)}$$

Where; TEFi is the toxicity equivalent factors for the carcinogenic PAHs

Ci is the concentrations of the individual carcinogenic PAHs in the samples

The MEQBAP was calculated using Equation 3.2

$$MEQBAP = \sum (MEFi \cdot Ci) \qquad \text{Eqn (3.2)}$$

Where; MEFi is the mutagenic equivalents factors for the mutagenic

3.11.1 Estimation of Dietary Intake

In this study, the concentrations of BaP and EFSA indicators were used to estimate the daily intakes of PAHs from oils and fried finger foods. The estimated daily intake (EDI) is given as

$$EDI\ (ngkg^{-1}\ bwday^{-1}) = \frac{C \times IngR}{BW} \qquad 3.3$$

IngR (g) represents the average daily consumption of vegetable oil. In grams, the average daily consumption of vegetable oil. Because there is no information on the average consumption rate of vegetable oil in Ghana, the Egyptian value of 20 g/day based on the Mediterranean average consumption pattern (Linseisen et al. 2002) was used. The average daily consumption of finger foods was 20.8 g 1). The average body weight (kg) (here assumed to be 60 kg for adults, 30Kg for adolescents, and 16 kg for children). C is the concentrations of EFA indicators for PAHs in food, namely, BaP, PAH2 (BaP + Chry), PAH4 (PAH2 + benzo[a] anthracene (BaA) and benzo[b] fluoranthene (BbF)) and PAH8 (PAH4 + benzo[k]fluoranthene (BkF), indeno[1,2,3-c,d]pyrene (IndP), dibenz[a,h] anthracene (D [a,h]A) and benzo[g,h,i]perylene (BghiP))

3.11.2 Margin of exposure

The margin of exposure (MOE) associated with consumption of these vegetable oil types and fried finger food was calculated using Equation 3.4.

$$MOE = \frac{BMDL_{10}}{EDI} \times 10^{6} \qquad \ldots\ldots\ldots\ldots (3.4)$$

BMDL10 denotes the lower bound of a 95% confidence interval for the benchmark dose

(BMD) that resulted in a 10% tumor incidence (European Food Safety Authority) (EFSA, 2008). EDI represents the estimated dietary intakes of BaP, PAH2, PAH4, and PAH8. The MOE value is calculated as follows: A MOE value of 10,000 denotes serious adverse health effects, while values greater than 10,000 denote no adverse effects on humans (European Food Safety Authority) (EFSA, 2008). The BMDL10 values (in mg kg 1 bw day 1) for the following PAH indicators are: BaP (0.07), PAH2 (0.17), PAH4 (0.34), and PAH8 (0.49) (European Food Safety Authority) (EFSA, 2008).

3.11.3 Hazard Index and Incremental Lifetime Cancer Risk

Equations 3.5 and 3.6 were used to calculate the non-cancer risk, expressed as the hazard index (HI), associated with the consumption of used oils and finger foods.

$$CDI_{ing-nc} = \frac{C \times IngR \times EF \times ED \times CF}{BW \times AT_{nc}} \quad \ldots\ldots (3.5)$$

$$HI = \frac{CDI_{ing-nc}}{RfD} \quad \ldots\ldots (3.6)$$

To further looked at an increased potential of acquiring cancer during a life time as a results of consumption of PAH contaminated with oil and finger foods, incremental lifetime cancer risk (ILCR) was calculated following the model equation of the US EPA (USEPA, 1989). The ILCR is given by Eq. (3.7):

$$ILCR = \frac{C \times IngR \times EF \times ED \times CF \times SFO}{BW \times ATnc} \quad \ldots\ldots (3.7)$$

where CDIing-nc is the chronic daily intake for PAH ingestion, EF is the exposure frequency (350 days per year), ED is the exposure duration in years (6 years and 30 years for children and adults, respectively), ATnc is the averaging time for non-carcinogens in days (2190 and 10,950 days for children and adults, respectively), and CF is a conversion factor (1x 10^6).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 PAHS CONTAMINATION IN UNUSED OIL

The three unused oils recorded seven PAHs (Nap, Ad, flr, Ant, Flu, Pyr, and B(b)F) out of the sixteen PAHs investigated. They were mostly low ring PAHs. Benzo(k)anthracene, which has five rings, was also detected in the fresh soya bean oil sample at a concentration of 13µg/kg. According to European Union Commission Regulation No. 127/2013, B(k)F is one of the carcinogenic PAHs, and it was the only carcinogenic PAH detected in the unused oils analyzed. Flu was found in varying concentrations in all three unused oil samples. The data of this study shows that soya bean oil accounted for the higher congeners of PAHs with five and mixed vegetable oil, sunflower oil registered 3 and 4 respectively. This could be because the mixed vegetable oil and soya bean oils were not subjected to thorough refining processes like sunflower oil. Deodorization processes were found to significantly reduce the levels of low molecular weight PAHs, while decolorization with activated charcoals significantly reduced the levels of high molecular weight PAHs (Larsson et al., 1987; Moret et al., 2005). The total concentration measured is 120µg/Kg with an average concentration of 16.85µg/Kg ± 21.5µg/Kg.

Anthracene registered the highest concentration value of 40µg/Kg in sunflower oil and Furanthene with lowest concentration in both mixed vegetable oil and sunflower oil as shown in Fig.4.1.

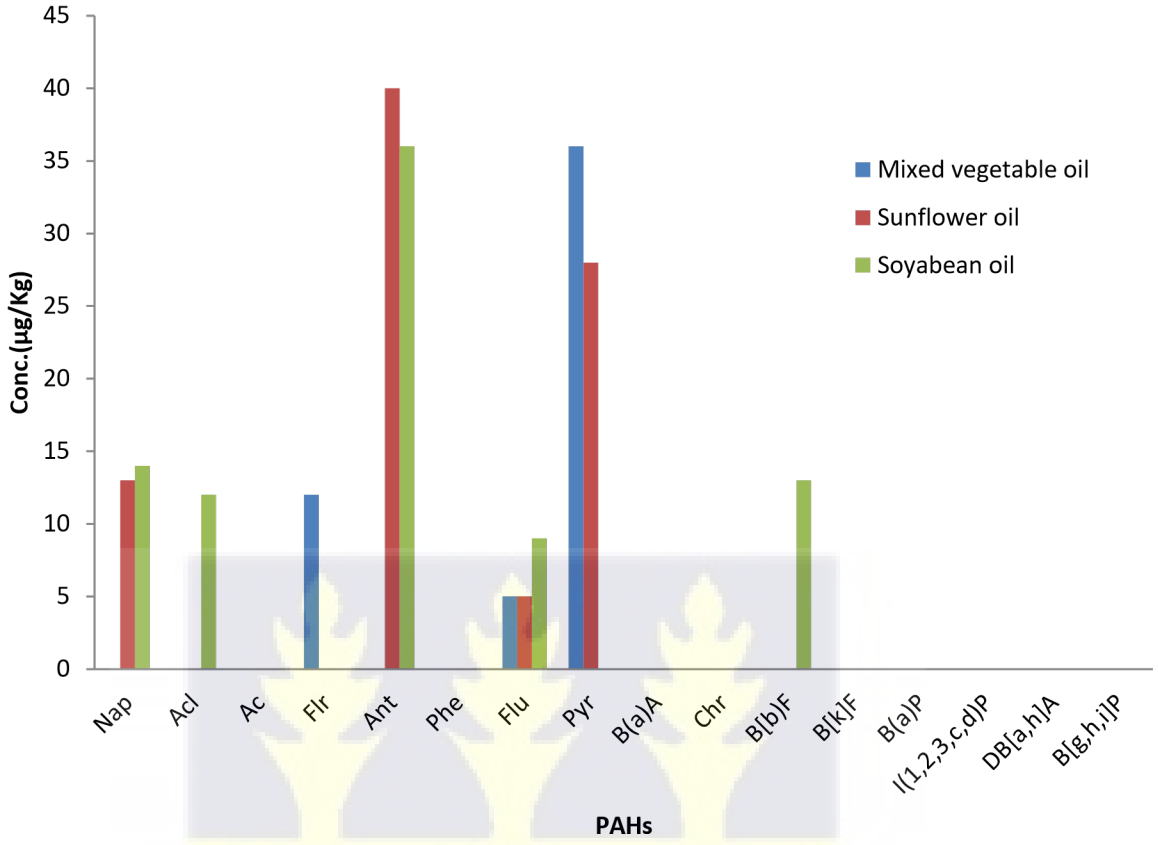

Figure 4. 1 PAHs Conc.(g/Kg) of the fresh oil types

The results from this study follow similar studies done in China (Yu et al., 2013 and Hao et al 2016) which studied four vegetable oil (rapeseed, soybean, peanut and olive oil) with sum concentrations of 2754.8µg/kg even though higher than this study. The PAHs found in the fresh oil samples were mostly low-ring PAHs (2- to 4-ring), correlating with the findings of previous studies in China by Wu et al, 2012, Ciecierska and Obiedzinski (2013), and Hao et al, 2016. This contamination can be attributed to air pollution, contaminated soil, or, in the case of imported edible oils, contamination at the source (Dost and deli,

2012). Moreover, PAH contamination in vegetable oils has been linked to a variety of processes, including drying and roasting prior to extraction (Moretti et al., 2016), solvent extraction, contamination from mineral or lubricating oils (Cejpek et al., 1998 and Sopelana, 2004), and further BaP migration from packaging materials (Bansal and Kim, 2015)

4.2 VARIATION IN TOTAL PAHS COUNT AND LEVELS DURING REPEATED FRYING OF EDIBLE OILS

Various compositions of the 15 registered PAHs were discovered during the frying process of the oils with various food items over three repeated fries. In general, PAH compositions and concentrations in oils rise with the number of fries, regardless of food type involved. This variable tendency is related with PAH buildup in cooking oils in the course of frying (Jin-Ku et al.,2021). The number of PAHs detected and their concentrations were shown in figure 4.2 to Figure 4.4 when sunflower oil was used to fry various food substances. When mixed vegetable oil was used to fry plantain chips, a total of fifteen PAHs were counted, with the majority of the HM-PAHs being recorded in the third fry session (Figure 4.5 to Figure 4.7 indicating that using mixed vegetable oil to fry plantain chips multiple times is not recommended. The HM-PAH buildup may be attributed to high temperatures and plantain chip manufacturing processes (Bansal and Kim, 2015).

Anthracene was found in all oil samples across the three stages of fry (Figure 4.8 to Figure 4.10 with high concentrations.

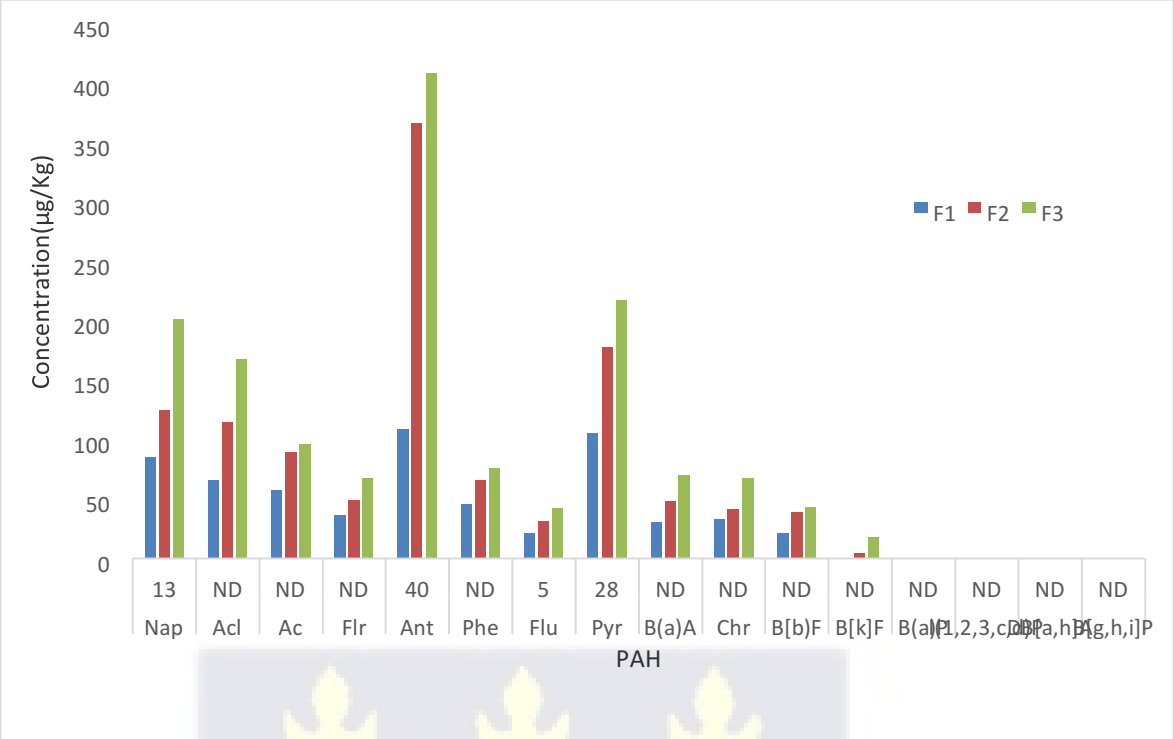

Figure 4. 2PAHs conc.(µg/Kg) of sunflower oil fried with beans cake

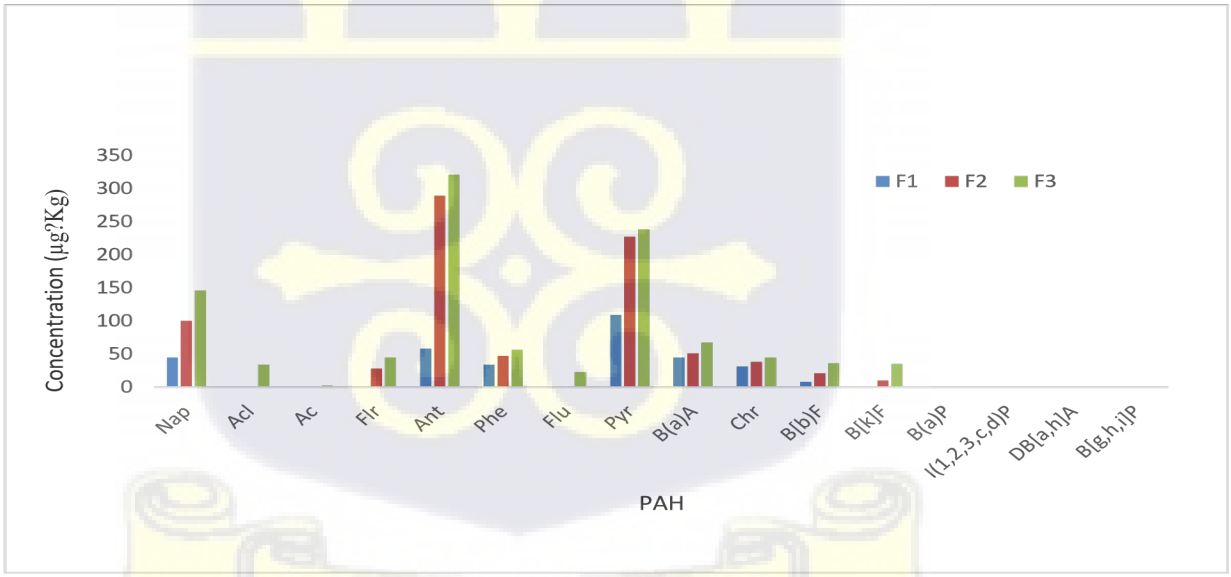

Figure 4. 3 PAH conc. (µg/Kg) in sunflower oil fried with plantain chips

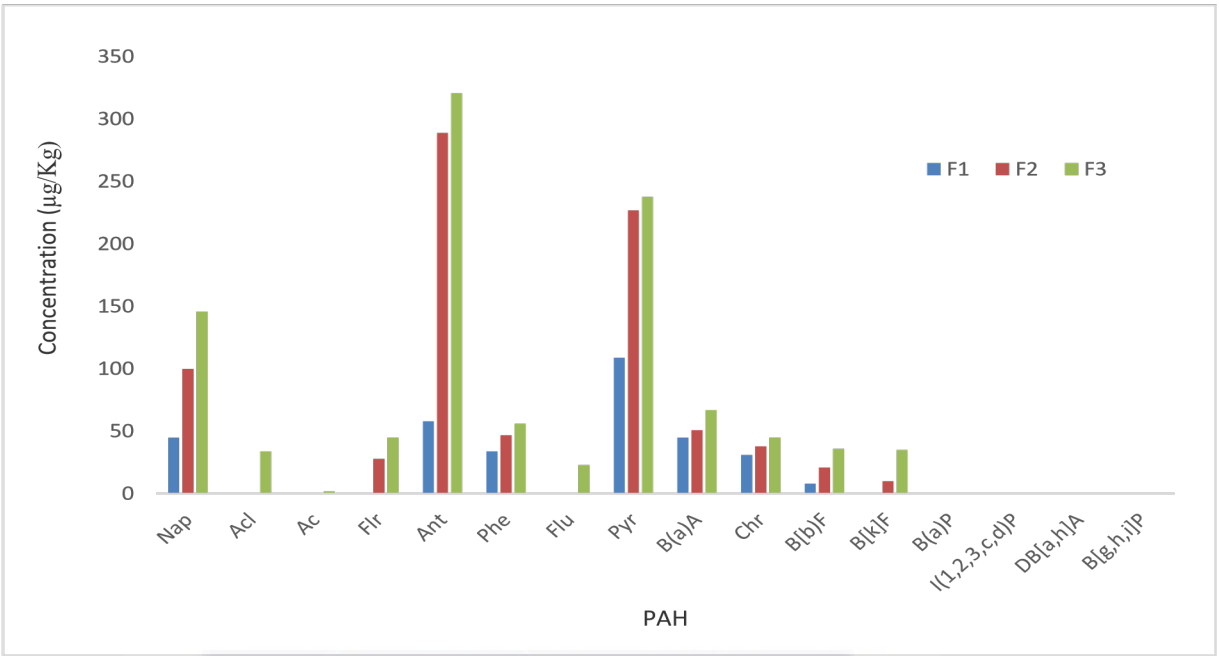

Figure 4. 4 PAH conc.(µg/Kg) in sunflower fried with doughnut

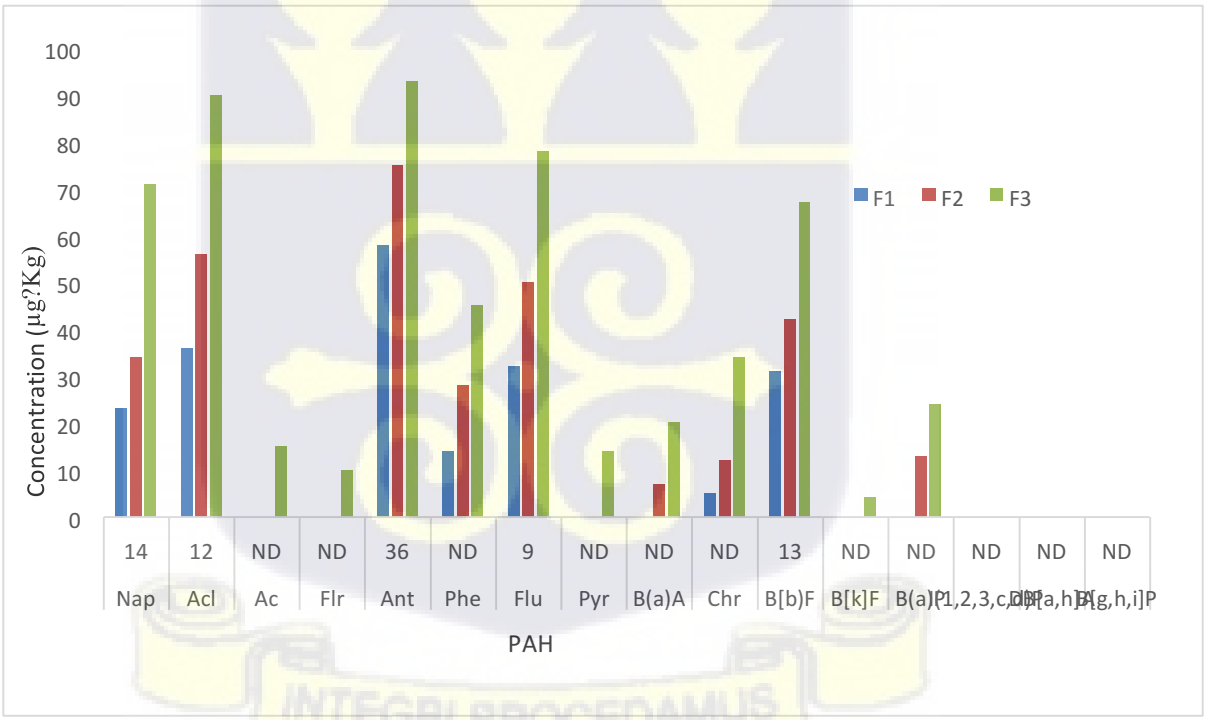

Figure 4. 5 PAH conc. (µg/Kg) in soya bean oil fried with beans cake

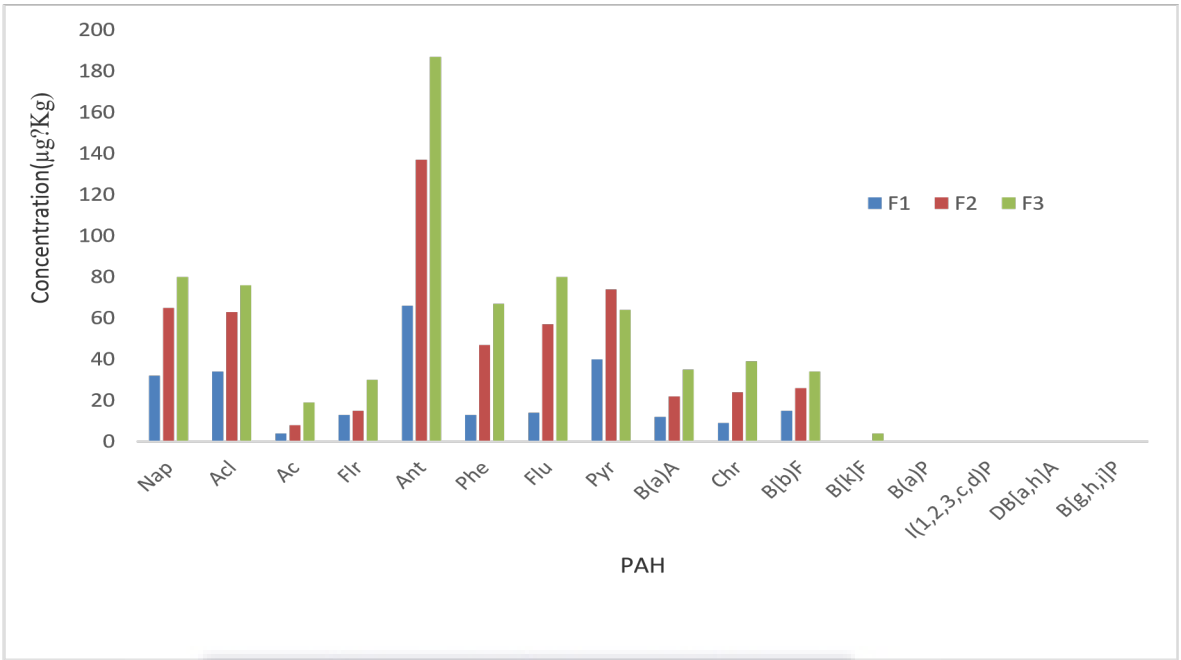

Figure 4. 6 PAH conc.(µg/Kg) In soya bean oil fried with plantain chips

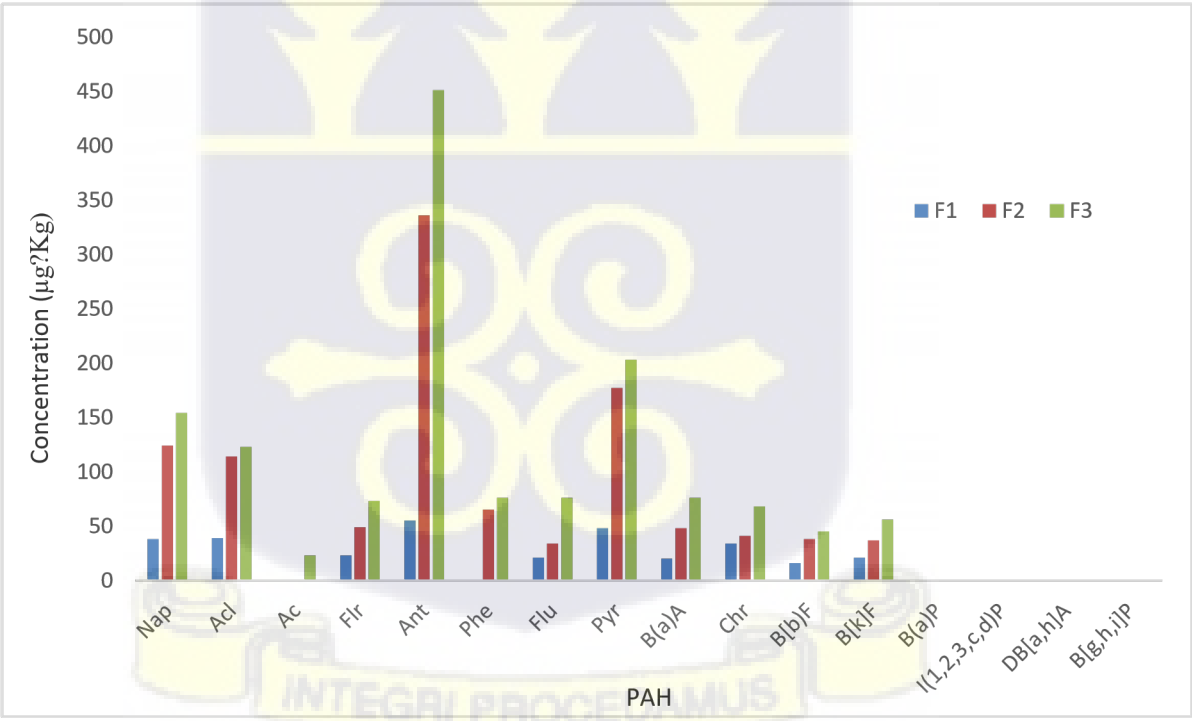

Figure 4. 7PAH conc.(µg/Kg) in soya bean oil fried with doughnut

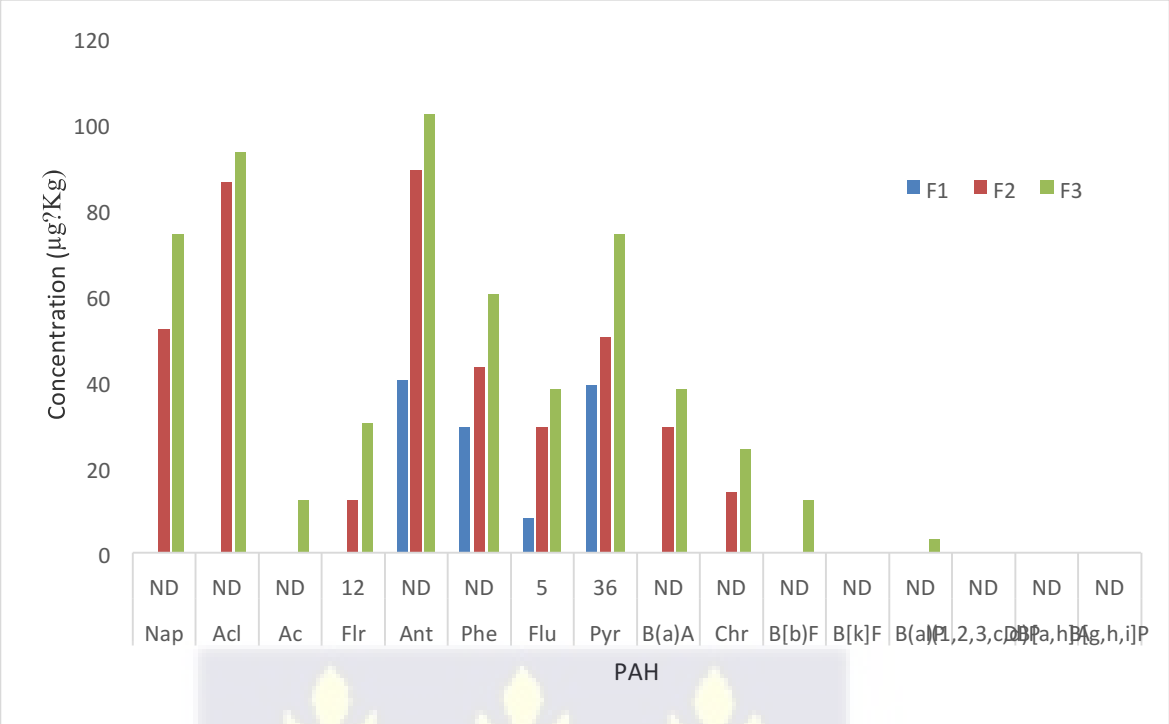

Figure 4. 8 PAH conc.(µg/Kg) in mixed vegetable oil fried with beans cake

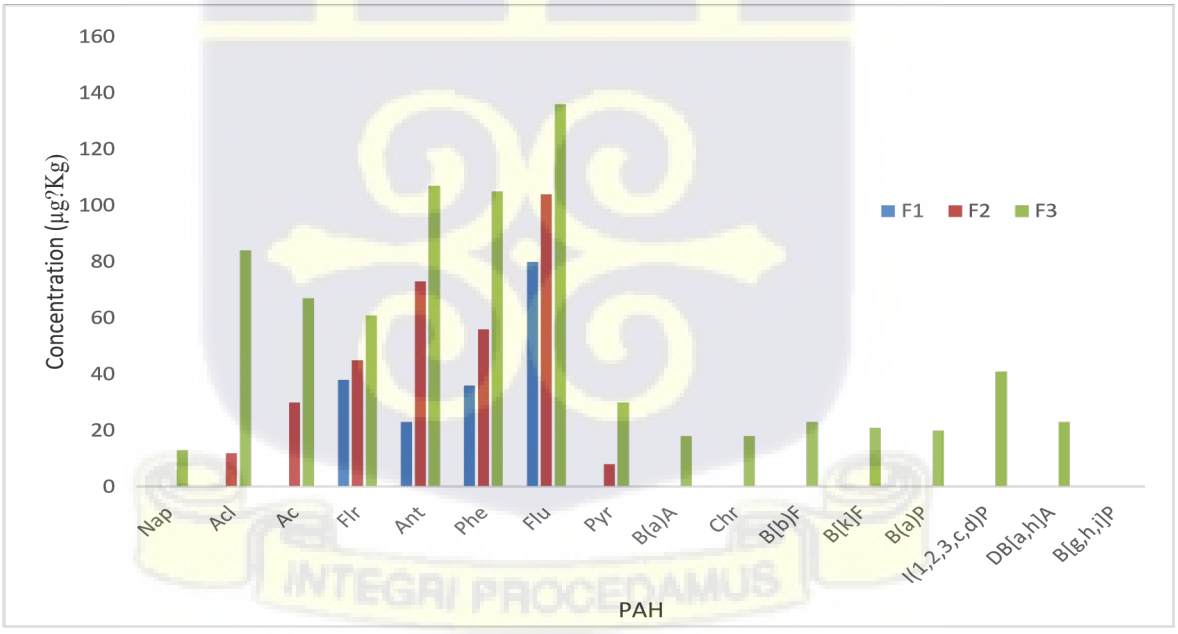

Figure 4. 9 PAH conc.(µg/Kg) in mixed vegetable oil fried with plantain chip

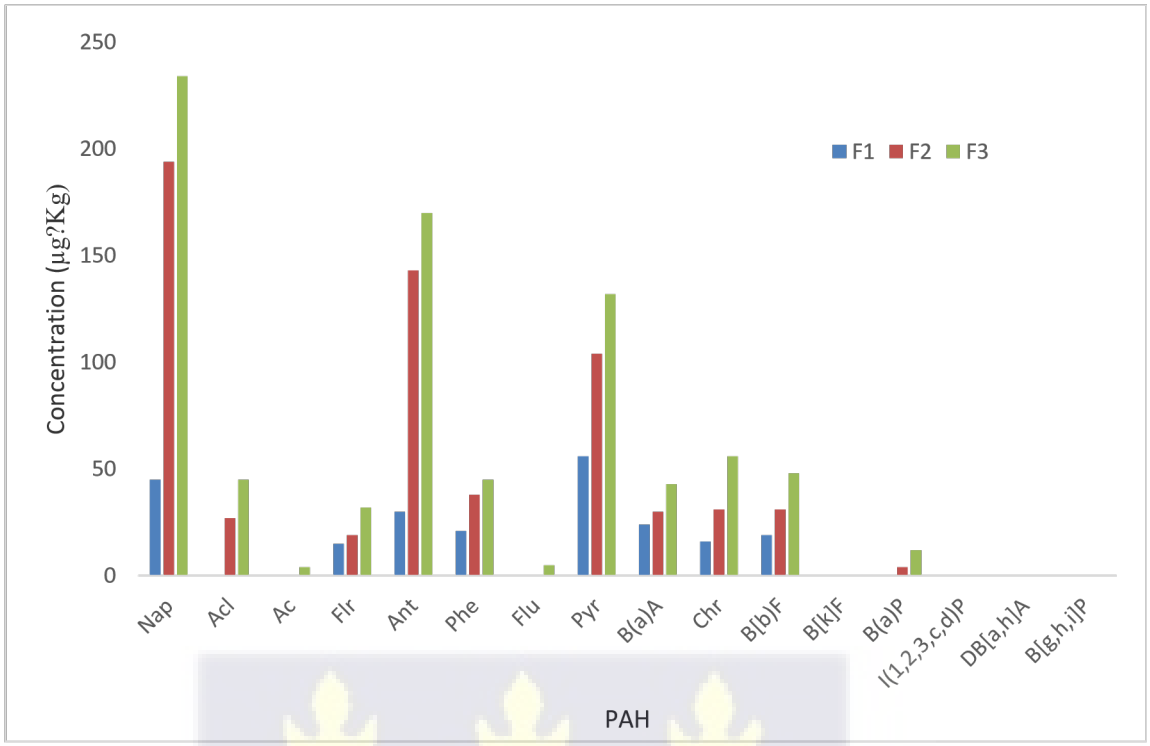

Figure 4. 10 PAH conc, (µg/Kg) in mixed vegetable oil fried with doughnut

Table 4. 1PAHs Concentration (ug/kg) of the edible oils

	Soya bean oil							Mixed vegetable oil										Sunflower oil									
	Beans cake				plantain chips			Doughnut			Beans cake				Plantain chips			Doughnut			Beans cake				Plantain chips			Doughnut		
	Low M-PAH																													
PAHs	F0	F1	F2	F3	F1	F2	F3	F1	F2	F3	F0	F1	F2	F3	F1	F2	F3	F1	F2	F3	F0	F1	F2	F3	F1	F2	F3	F1	F2	F3
Nap	14	23	34	71	32	65	80	38	124	154	ND	42	52	74	ND	ND	13	45	194	##	13	85	124	201	18	34	41	45	100	146
Acl	12	36	56	90	34	63	76	39	114	123	ND	ND	86	93	ND	12	84	ND	27	45	ND	65	114	167	ND	ND	3	ND	ND	34
Ac	ND	ND	ND	15	4	8	19	ND	ND	23	ND	ND	ND	12	DN	30	67	ND	ND	4	ND	57	89	96	ND	3	10	ND	ND	2
Flr	ND	ND	ND	10	13	15	30	23	49	73	12	ND	12	30	38	45	61	15	19	32	ND	36	49	67	ND	12	20	ND	28	45
Ant	36	58	75	93	66	137	187	55	336	451	ND	40	89	102	23	73	107	30	143	170	40	109	336	408	46	62	65	58	289	321
Phe	ND	14	28	45	13	47	67	ND	65	76	ND	29	43	60	36	56	105	21	38	45	ND	45	65	76	ND	1	13	34	47	56
	Medium M-P																H													
Flu	9	32	50	78	14	57	80	21	34	76	5	8	29	38	80	104	136	ND	ND	5	5	21	31	42	10	15	24	ND	ND	23
Pyr	ND	ND	ND	14	40	74	64	48	177	203	36	39	50	74	ND	8	30	56	104	132	28	105	177	217	ND	ND	ND	109	227	238
B(a)A	ND	ND	7	20	12	22	35	20	48	76	ND	ND	29	38	ND	ND	18	24	30	43	ND	30	48	70	ND	ND	4	45	51	67
Chr	ND	5	12	34	9	24	39	34	41	68	ND	ND	14	24	ND	ND	18	16	31	56	ND	32	41	67	20	36	45	31	38	45
	High M-PAH																													
B[b)F	13	31	42	67	15	26	34	16	38	45	ND	ND	ND	12	ND	ND	23	19	31	48	ND	21	38	43	ND	ND	ND	8	21	36
B[k]F	ND	ND	ND	4	ND	ND	4	21	37	56	ND	ND	ND	ND	ND	ND	21	ND	ND	ND	ND	ND	4	17	ND	ND	ND	ND	10	35
B(a)P	ND	ND	13	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	ND	20	ND	4	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
I(1,2,3,c,d)P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DB[a,h]A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B[g,h,i]P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sum	84	199	317	565	252	538	715	315	1063	1424	53	158	404	560	177	328	767	226	621	592	86	606	1116	1471	94	163	225	330	811	1048
2ring	14	23	34	71	32	65	80	38	124	154	0	42	52	74	0	0	13	45	194	0	13	85	124	201	18	34	41	45	100	146
3 +4 rings	45	109	172	294	167	376	502	201	750	1023	53	116	266	366	177	286	475	162	365	483	73	378	747	947	76	126	171	277	680	795
5+6 rings	13	31	55	95	15	26	38	37	75	101	0	0	0	15	0	0	128	19	35	60	0	21	42	60	0	0	0	8	31	71
2PAH	0	5	25	58	9	24	39	34	41	68	0	0	14	27	0	0	38	16	35	68	0	32	41	67	20	36	45	31	38	45
4PAH	13	36	74	145	36	72	108	70	127	189	0	0	43	74	0	0	59	59	92	147	0	83	127	180	20	36	49	84	110	148

4.3 EFFECT OF DEEP FRY SESSIONS ON COMPOSITION AND CONCENTRATION OF PAHS IN EDIBLE OIL

The concentrations of 16PAHs in the oil samples in this study ranged from LOD - 6020 µg/kg (Table 4.2) when compared to catering services in Beijing, China, where these values ranged from 3750 µg/kg to 7590µg/kg (Hao et al., 2016b). The concentrations and compositions of the 16 PAHs changed significantly after repeated frying of edible oils, which varied depending on the oil type. After three frying sessions, for example, the 16 PAH concentration in soybean oil increased from 84 to 5472 µg/kg. Both sunflower and mixed vegetable oils had higher levels of 16 PAHs (Tables 4.1). Changes in PAH concentrations and proportions may be attributed to the interaction of long deep frying times, cyclization, and the transformation of LMW PAHs into HMW PAHs as the frying temperature rises (Jin-Ku et al.,2021). PAH concentrations and compositions varied depending on the oil type. Sunflower oil produced lower PAH concentrations when fried with Plantain chips. However, high concentrations were found when plantain chips were fried in mixed vegetable oil, implying that sunflower oil may be a good choice for frying plantain chips (Fig. 3a). Similarly, beans cake had lower concentrations when fried in soya bean oil than when fried in sunflower oil (Fig.3c). Each oil has a different smoking point, and smoking point indicates that the oil is being broken down, which may result in the release of unwanted compounds like PAHs (Eyres 2015).

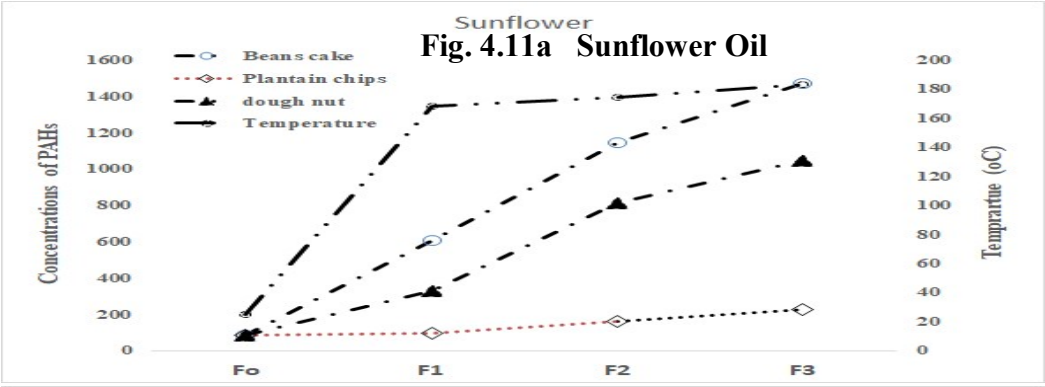

Fig. 4.11a Sunflower Oil

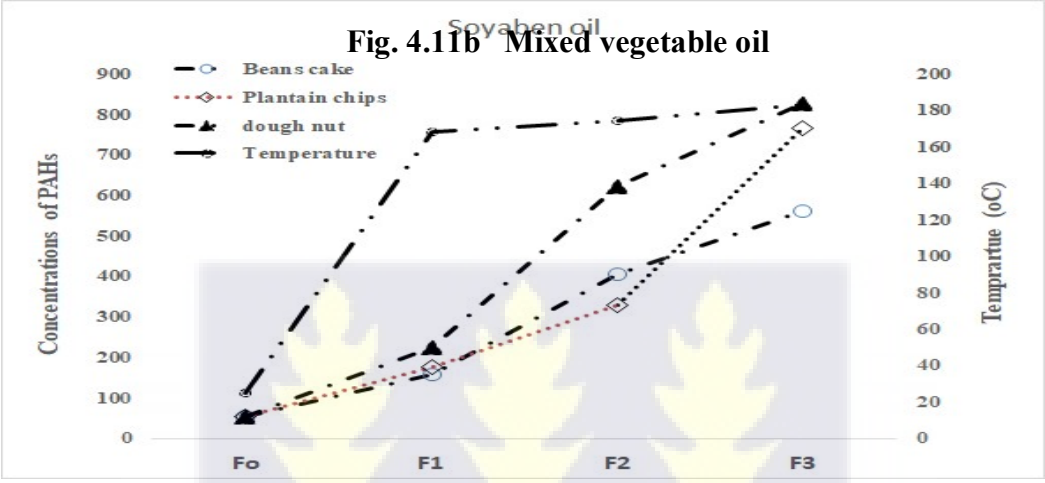

Fig. 4.11b Mixed vegetable oil

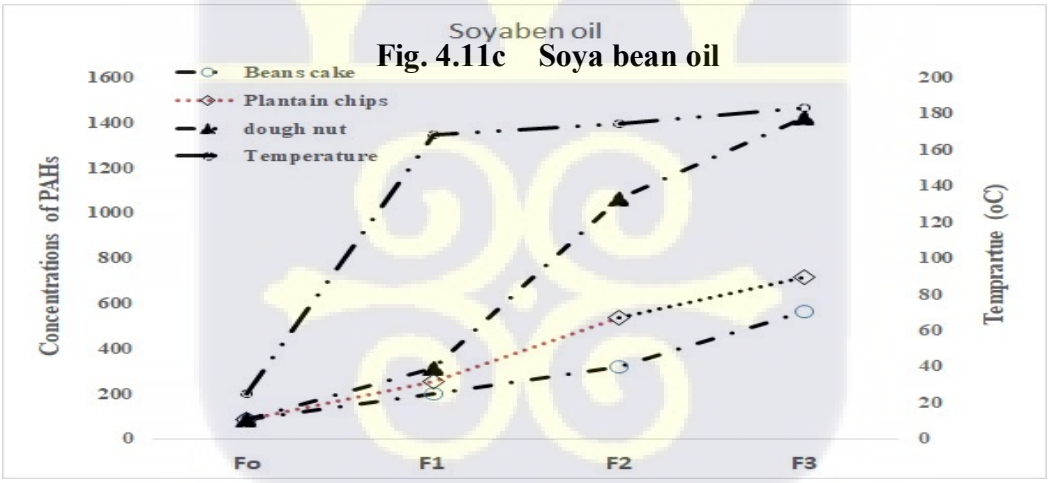

Fig. 4.11c Soya bean oil

Figure 4. 11 shows the variation of concentration with temperature (a) sunflower oil, (b) mixed oil and (c) soya bean oil

Table 4. 2 Total concentrations (µg/kg) in all the samples

Samples	Concentration (µg/kg)
Sunflower oil	6020
Soya bean oil	5472
Frytol oil	4120
Food	1827

This explains why different types of oil produce different levels of PAHs. Again, food recipe was discovered to have an effect on PAH formation during frying (Li et al. 2016)

The appearance of significant amounts of Flt and Pyr in some crude oil is a cause for concern since these compounds, when combined with BaP, are thought to be carcinogenic. Stepanov et al., 2010; Effiong et al., 2016). The concentrations of Flt in the mixed vegetable oil fried with beans cake were below the detection limit in the unused oil and third fry, respectively, and 30µg/Kg and 36-74 µg/Kg of pyrene were measured in the unused oil and third fry, with a similar pattern observed for other oil components. This could be due to LMPAHs compound transformations via cyclization reactions at higher frying temperatures. Changes in frying intensity, temperature, and time, as well as significant differences between PAHs during frying, all contribute to variation in PAH concentrations between frying sessions (An et al., 2017). We meticulously replicated typical food vendor conditions in this study, in which frying temperature and length of time were not held in check and frying completeness was determined solely by thorough observation. During the

various frying sessions, the masses of finger foods were not exactly the same; frying heavier samples takes longer than frying lighter samples

Repolymerization produces radicals, which are then recombined to form larger and more stable molecules such as PAHs via pyro synthesis. This controls how PAHs behave between the initial concentration and subsequent frying sessions. Temperature increases may be the cause of these effects. This could have helped to convert low molecular weight PAHs to high molecular weight PAHs. All samples' PAH compositions gradually increased over repeated fry sessions, following the pattern F0<F1<F2<F3. The increases could be attributed to the fuel used, high temperatures, and long deep-frying times (Teixeira et al., 2007; Wu & Yu., 2012). The percentage increases in oil samples from unused oil (F0) to third frying sessions (F3) were: 20.5% for sunflower with beans cake, 48% for mixed vegetable oil, and 18% for soya beans oil with dough nut. The varying percentage increases can be attributed to the different oil types, temperature variations, and frying session duration. Even though the PAH congeners distribution patterns demonstrated that LMPAH PAHs outnumbered HMPAHs at each frying cycle. During the frying cycles, the homologues' composition changed dramatically, with mean concentrations of LMPAH, MMPAH, and HMPAH in sunflower oil being 82.7 µg/Kg, 62.6 µg/Kg, and 23 µg/Kg, respectively (Table 4.3)

Table 4.3 Total mean concentration (µg/Kg) of PAHs

Oil type	LM-PAHs	MM-PAHs	HM-PAHs
Sunflower oil	82.7	62.6	23
Soya bean oil	68.2	46.4	28.6
Frytol oil	58.8	43.9	21.4
Food	62.4	17.7	00

4.4 DISTRIBUTION OF LPAH, MPAH, HPAH, PAH4, AND BAP CONCENTRATIONS IN EDIBLE OILS

PAHs are categorized depending on the number of aromatic rings they contain; those with 2-4 aromatic rings are known as Light Molecular weight (LM) PAHs, while those with 56 aromatic rings are referred to as Heavy Molecular Weight (HM) PAHs (Chen et al. 2012 and Hao et al., 2016). According to Hao et al. (2016b), Chen et al. (2012), and Li et al. (2003), PAH toxicity levels are determined by the number of rings, with more rings being more toxic. Irrespective of the cooking oil, food product, or number of frying sessions used, the principal PAHs found in the samples were LPAH and MPAH (Nap, Ac, Acl, Flr, Phe, Ant, Chr, B(a)A, and Flu) with an average detection percentage of 85.8% (88.5% sunflower oil, 86% mixed vegetable oil, and 83% soya bean oil). However,

composition of HM-PAHs in all the samples increased with increasing frying sessions echoing previous studies by Dennis et al., (1991), Wu et al., (2012) and Hao et al., (2016). High molecular PAHs were detected in various concentration whiles frying with the oils. However, B(a)P, I(1,2,3,c,d)P, DB[a,h]A and B[g,h,i]P (MH-PAHs) were absent in sunflower oil samples fried with various food items. (B[b)F and B[k]F) recorded the highest concentration of 43µg/Kg and lowest of 8µg/Kg respectively. In all samples, Benzo(g,h,i)Pyrene was below the detection limit. The third fry of plantain chips with I(a1,2,3,cd)P and DB(a,h)A mixed vegetable oil measured 41µg/Kg and 23µg/Kg, respectively (Table 2). I(1,2,3,c,d)P, DB[a,h]A, and B[g,h,i]P were detected in samples of soya bean oil used to fry various food items, but the rest of the HM-PAHs recorded different concentrations across the different number of fries. The proportion of higher molecular PAHs found among all PAHs increased depending on the number of times the oil is used and varied for different foods. HMPAHs may have formed as a result of high temperatures, oil type, fuel type, and food ingredients. R elatively high HM-PAH level indicates that oil which has been used to fry food continually is more dangerous to human health than fresh oil, which is consistent with the findings of a study conducted in China by Hao et al,. 2016.

Soya bean oil (table 2) shows 49.5% detection for LM-PAHs, while MM-PAHs and HMPAHs recorded 33.6% and 16.8%, respectively. Mixed vegetable oil registered 51.7%, 34%, and 14%, for LM, MM, and HM PAH respectively. Sunflower oil had the highest percentage detection of LM-PAHs with a score of 52.8%. Food items showed no or below detection for the HM-PAHs, but 25% was measured for the LM-PAHs in the food. The soya bean brand was found to have the highest overall count of 15 PAHs, with 101 PAHs

been detected throughout multiple fry with the food implying its repeated use may cause serious health concerns to the public. Whatever the food item being fried, the overall pattern observed in terms of number of detections and concentrations were LMPAHs <

MPAHs < HMPAHs which was in line with similar findings from other parts of the world (Rose et al., 2015, Abdel-shafy et al., 2016 and Jin-Ku et al., 2021)

The total PAH4 (Chr, B(a)P, B(b)F, and B(a)A) after multiple frying sessions was 75 µg/Kg, 352 µg/kg and 588 µg/kg for soya bean, mixed vegetable and sunflower respectively, according to the study's findings. These values exceeded the recommended value of 10 µg/kg by EU Regulation No. 836/2011.

Because B(a)P contributes significantly to the overall carcinogenic potential, it is frequently employed as a pointer for overall PAH exposure. The B(a)P levels in bean cakes after the second and third fryings with soya bean oil were 13 and 24 µg/kg, respectively. Again, the third fry of mixed vegetable oil with bean cake and plantain chips was at levels the of 3 and 20 µg/kg, respectively. These levels were substantially higher than the EU Regulation 837/2011 recommended maximum limit of 2 µg/kg. Because they exceed the recommended maximum limit, this may cause health concerns. This study found that repeated oil use produced B(a)P, which is consistent with the findings of similar studies published by Darwish et al. (2019).

B(k)F, I(1,2,3,cd), and DB(a,h)A concentrations in mixed vegetable oil samples fried with plantain chips were 21µg/Kg, 41µg/Kg, and 23µg/Kg, respectively (Table 2). B(k)P was

also found in sunflower oil samples in the beans cake second and third fries, with 4 µg/Kg and 17 µg/Kg, respectively, and dough nut with 10µg/Kg and 35µg/Kg, respectively. According to the study, carcinogenic PAHs concentrations increase with repeated use of the oil, which was also observed in Li et al. 2003, See et al. 2006, Abdullahi et al. 2013 and Jin-Kui et al 2021 investigations in edible oils. The elevated levels of benzo(a)pyrene, PAH4, and other highly toxic PAHs in the samples could be attributed to uncontrolled food processing temperatures and the type of fuel used in preparation. Rose et al., (2015) reported on different PAH levels for various fuels used, and Abdel-shafy et al., (2016) reported that food cooked at high temperatures via grilling, roasting, or frying is a significant source of PAHs. Furthermore, PAH contamination in vegetable oils may be linked to a variety of processes, including drying and roasting prior to extraction (Moretti et al., 2016), solvent extraction and further transfer of BaP from packaging materials (Bansal and Kim, 2015)

4.5 PAHS LEVELS AND COMPOSITION IN FINGER FOODS

Various concentrations of low- and medium-ring PAHs were detected; however, HM PAHs were below the detection limit, whereas Ant and Nap showed 100% detection (Table 5). The average PAH concentration in processed but unfried plantain chips was 27.50 µg/Kg, doughnuts 18.44 µg/Kg, and beans cake 20.94 µg/Kg, all of which were higher than the 13.8 µ g/kg found by Iwegbe et al., 2020, when fish were repeatedly fried with six different oil types. Fried food items generally showed an increase in concentration after a series of frying sessions; for example, the highest Nap concentration (776 µg/kg) for this study was

recorded for fried plantain chips, representing a 77.7% increase over fresh plantain chips. The ingredients, fuel source, and processing of plantain chips may have all contributed to the astronomical increase in concentration (Li et al. 2016). The concentrations and composition of 16 PAHs in various foods varied, but were lower than in oil samples analyzed in the same study. Because PAHs are highly lipophilic, plantain chips, donuts, and bean cakes contain fewer lipids, resulting in lower detection and concentration levels, especially for HM-PAH. The concentrations of BaP and HM-PAH in processed, unfried food samples were below their respective detection limits, but the concentration of 4PAH in the same food substances was 54 µg/kg. The presence of 4PAHs can be hazardous to one's health because they have been shown to be more toxic.

4. 6 CARCINOGENIC PAHS IN THE EDIBLE OILS

According to European Commission Regulation No. 836/2011, the maximum limit for PAH4 in edible oil and fats should be 10µg/kg and 2gµ/kg for B(a)P. In this study, the PAH4 (B(a)A, Chr, B[b]F, and B(a)P) concentrations, which are considered carcinogenic, far exceeded the maximum limit in all of the used oil samples fried with the three different foods. Because of its high contribution to total carcinogenic potential, benzo[a]pyrene (B[a]P) is frequently used as a pointer for total exposure to carcinogenic PAHs (Darwish et al. 2019). In this study, Benzo(a)pyrene was found in the used oil samples at concentrations far exceeding the maximum. In mixed vegetable oil samples third fries with beans cake, plantain chips and dough nut, 3µg/Kg, 20µg/Kg and 12µg/Kg respectively was recorded and that of the soya bean sample fried with beans cake measured 13µg/Kg and

24µg/Kg for second and third fries respectively, repeated use of oil was found to generate B(a)P which agrees with the similar studies done in China by Li et al. 2003, Hao et al, 2016 and Darwish et al. 2019. The other known carcinogenic PAHs that were detected in this study were in the third fry using frytol vegetable oil sample with plantain chips B(k)F, I(1,2,3,c,d) and DB(a,h)A with concentrations 21µg/Kg, 41µg/Kg and respectively (Table 5). B(k)P was recorded in the second and third fries sunflower oil samples fried with both beans cake and dough nut, in the beans cake second and third fries recorded 4 and 17 respectively whiles in dough nut 10µg/Kg and 35µg/Kg respectively were measured. The study revealed that, carcinogenic PAHs concentrations increase with increasing repeated use of the oil and this was same for Li et al. 2003, See et al. 2006, Abdullahi et al. 2013 and Jin-Kui et al 2021 investigations in edible oil.

4. 7 COMPOSITION AND CONCENTRATION OF PAHS IN FINGER FOOD

Various low and medium ring PAH concentrations were detected, but HM PAHs were below the detection limit, whereas Ant and Nap showed 100% detection (Appendix VIII). The average PAH concentration in processed but unfried plantain chips, doughnut, and bean cake was 27.50 µg/ kg, 18.44 µg/ kg, and 20.94 µg/ kg, respectively, which was higher than the 13.8 µg/ kg recorde by Chukwujindu et al., 2020 when fish was repeatedly fried with six different types of oil. Fried food items generally showed an increase in concentrations after several frying sessions; for example, the highest concentration of Nap (776µg/kg) for this study was recorded for fried plantain chips, representing a 77.7% increase over fresh plantain chips. The ingredients, fuel source, and plantain chip

processing could all have contributed to the astronomical increase in concentration (Li et al. 2016). PAHs are formed at high temperatures as a result of the thermal breakdown of certain food constituents such as amino acids, fatty acids, steroids (e.g., cholesterol), and tri glycerides (Chen et al., 2001; Sharma et al., 2003; Hao et al., 2016a). In all of the food samples tested, LMW PAHs (2-3 rings) outnumbered MM PAHs (4 rings). The following homologue distribution was found in all food samples: 2-ring PAHs are followed by 3-ring PAHs, 4-ring PAHs, 5-ring PAHs, and 6-ring PAHs. The concentrations and composition of 16PAHs in different foods differed, but they were lower than in oil samples analyzed for the same study. Because of the high lipophilic nature of PAHs, plantain chips, doughnuts, and bean cake contain fewer lipids and thus lower detection and concentrations. BaP and HM-PAH concentrations in processed unfried and fried food samples were below their various limits of detection, but 4PAH concentration in the same food substances was 54 µg/kg. Because 4PAHs have been shown to be more toxic, their presence may be hazardous to one's health.

4.8 NON- CARCINOGENIC AND CARCINOGENIC PAHS IN THE EDIBLE OILS SAMPLES

The findings of this study revealed that non-carcinogenic PAHs were more prevalent than carcinogenic PAHs (Fig.4.12). However, with increasing frying, there was an increase in carcinogenic PAHs. Carcinogenic PAHs, either individual or mixture have been shown to have high toxic effect on cellular protein and DNA when metabolites, such as epoxides and dihydrodiols bind with these protein products (Arey et al., 2003). Mutations, developmental malformations, tumors, and cancer are caused by biochemical disorders and cell damage. PAH4 detection was high in the third fry of plantain chips with mixed vegetable oil with ΣPAH4 concentration of 61µg/kg six times higher than recommended limit in edible oil and fats set by EU (EU Regulation no 836/2011). Again, after the third fry of plantain chips with mixed vegetable oil, PAHs that are carcinogenic have been identified. Benzo (a) Pyrene (LOD-2030µg/kg), Indeno(1,2,3,c,d) (LOD-30µg/kg Benzo(a)Anthracene (LOD-18µg/kg), Benzo(b)fluoranthene (LOD-23µg/kg),

Benzo(k)fluoranthene (LOD-21µg/kg) (Table 4. 2). Whiles Indeno(1,2,3-c, d) recorded at 12µg/kg, 37µg/kg, and 41µg/kg in the first, second, and third fry respectively.. This is an indication that repeated fry of the edible oil encourage formation of the carcinogenic PAHs and it collaborated the work by Xuewei et al., 2016

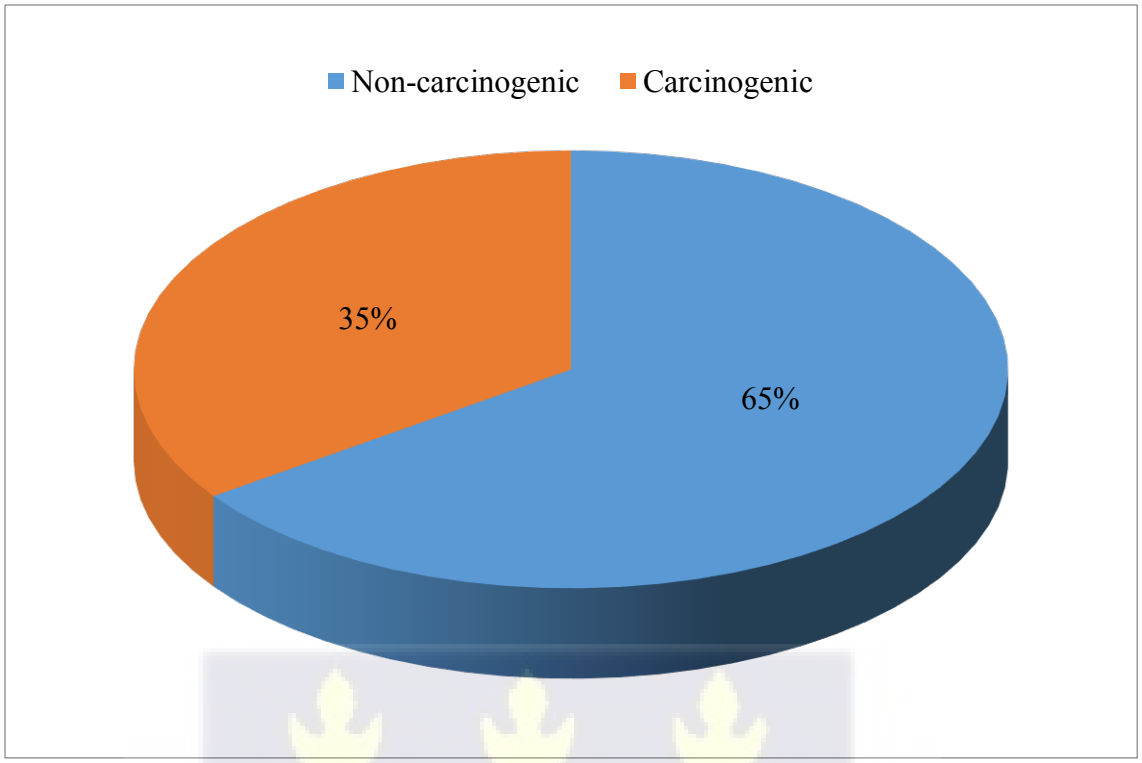

Figure 4. 12 Distribution of Carcinogenic and Non-carcinogenic PAHs

4.9 ANALYSIS OF VARIANCE (ANOVA) TEST FOR PAHS CONCENTRATIONS IN EDIBLE OIL

Appendix I display the output of an ANOVA test on the PAH Concentrations in Soybean Oil. The test revealed that there is a statistical P-Value of 0.0001 difference in the averages of the PAHs Concentrations in Soybean Oil at 5% significant level. There was the need to carry out a post-hoc test to determine the groups(PAHs) in which statistical significant difference really existed as display in appendix II. From appendix II it was observed that, the concentration in Ant is more than Nap, Ac, Flr, Flu Pyr, BaA, Chr, BaF, BaP,

I(123cd)P, DBahA and BghiP at 5% significant value.

Similarly, ANOVA test on the PAHs Concentrations in Sunflower Oil (appendix III), revealed that, there is a statistically significant P-Value=0.0001 difference in the averages of the PAHs Concentrations in Sunflower Oil at 5% significant value. The further test in differences in mean of PAHs shows statistically significant difference in means. There is more concentration in Ant than that of Nap, Acl, Ac, Flr, Phe, Flu, BaA, Chr, BaF, BkF, BaP, I123cdP, DBahA and BghiP. Nap has also shown a more PAH concentration than BaP, I123cdP, DB(ah)A and B(ghi)P and finally from appendix IV Pyr has also shown a more PAH concentration than Flr, Flu, BaF, BaP, I123cdP, DB(ah)A and B(ghi)P. Trend follows in the mixed vegetable oil where statistical P-Value was 0.0001 difference in the averages of the PAHs Concentrations at 5% significant value.

4.10 SOURCES OF PAHS

The PAH isomer pair diagnostic ratio of the entire sample results was calculated to determine yet if the PAHs recorded in the samples were due to external pollution or contamination from processing techniques. Other researchers, Essumang et al., 2012 described diagnostic tools for tracing the source of PAHs that take advantage of the fact that different sources of PAHs have different molecular compositions

Table 4.3 displays the results of the PAH diagnostic ratios. The following values were obtained: AN/AN+PH 0.50, FL/FL+PYR

0.50, BAP/BAP+A 0.62, IND/INDP+BGHI 0.50, NAP/NAP+ACL 0.32, and Ind/Ind+Dib

0.49. The results agreed with Rose et al. (2015) of pyrolytic or pyrogenic source values. This may be due to the finger foods being deep fried at elevated temperatures for an extended period of time. Rose et al. (2015 discovered that frying foods can introduce more PAHs, and that longer frying times are associated with the formation of more PAHs in food.

Table 4. 3 Pair isomer diagnostic ratio

PAH Ratio	This Study	Petrogenic	Pyrogenic/Prolytic	Remark
AN/AN+PH	0.50	<0.5	>0.50	
FL/FL+PYR	0.50	-	>0.50*	
BAP/BAP+A	0.62	Up 0.35	>0.35	
IND/INDP+BGHI	0.50	< 0.2	0.2-0.5	
NAP/NAP+ACL	0.32	<0.10	>0.10	
Ind/Ind+Dib	0.49	0.2-0.35	0.2-0.35	Mixed Petro/Pyro

Adopted from Essumang et al., 2009

4.11 HUMAN HEALTH RISK ASSESSMENT

The possible health risk associated with the consumption of the utilized oils and fried finger foods was evaluated using a variety of methods. The seven (7) PAHs known to be carcinogenic and mutagenic (US EPA 2002) were used to calculate BaP toxicity equivalency

4.11.a BaP toxicity

The BaPTEQ and BaPMEQ levels of the seven PAHs in edible oils after three frying sessions. Only B(b)F in soya bean oil contributed to the 1.3 µg/kg BaPTEQ concentration in unused oil. This value is lower than the 4.5-116 µg/kg reported by Iwegbue et al., (2020) for unused oils. The BaPTEQ concentrations in these oils were generally higher after the third frying session than in the other frying cycles and unused vegetable oils. The BapTEQ values for oil samples after frying sessions ranged from 1.85µg/kg to 129.30µg/kg, with the maximum BaPTEQ values for soya bean, mixed vegetable, and sunflower oil being 144µg/kg, 103µg/kg, and 148µg/kg, respectively. The PAH BaPTEQ figures in oils samples were higher than in previous Nigerian studies by Iwegbue et al (2019), Iwegbue et al (2020), and An et al (2017). After the third frying session, all oil samples had higher BaPTEQ values. For instance, the maximum BaPTEQ values for third frying cycles for soya bean, mixed vegetable and sunflower oils are 24µg/kg, 3.8µg/kg and 7µg/kg respectively (Supplementary Table S2The BaPMEQ concentrations followed the same pattern as the BaPTEQ concentrations, with values ranging from 16.75 µg/kg in soya bean oil to 4.1 µg/kg in mixed vegetable oil and 10.75 µg/kg in sunflower oil. The BaPTEQ and BaPMEQ values in this study are dominated by B(a)A, B(a)P, and B(b)F, indicating that these carcinogenic PAHs must be urgently monitored by the Ghana Food and Drug Authority, who must immediately implement contamination mitigating plans to prevent cancer in individuals who regularly consume foods prepared with such oils. The BaPTEQ and BaPMEQ values of the fried finger foods varied from 6 µg/kg to 20 µg/kg and 0.14 µg/kg to 1.04 µg/kg respectively. BaPTEQ values was higher than 0.56 µg/kg to 7.80 µg/kg

reported by Iwegbue (2020) but lower for BaPMEQ value of 0.71 µg/kg to 2.59 µg/kg when fish was fried for the same investigation.

4.11.b Estimation of dietary intake

In comparison to similar studies by Iwegbue et al., (2019), children's dietary intakes of PAHs from these oil types as food ingredients ranged from 0.0 to 48.75 ng BaP kg/bw/day, 14.6-443.75 ng PAH2 kg/bw/day, 29.16-1087.5 ng PAH4 kg/bw/day, and 29.16-124 ng PAH8 kg/bw/day. The calculated daily intake figures for edible oil used three times to fry various finger foods shows higher values The daily intake value of mixed vegetable oil of 48.75ng BaP Kg/bw/day suggests that its use as an ingredient in food after three rounds of frying is more harmful to health than sunflower oil, which has no health effect (Appendix V). Adolescent and adult estimated daily intakes follow a similar pattern, with the highest daily intakes of 26 ng BaP Kg/bw/day and 12.3 ng BaPKg/bw/day, respectively. Children have serious health concerns based on their estimated daily intake of soya bean and mixed vegetable oils as food ingredients for all PAH categories except sunflower oil. This suggests that after repeated frying with finger foods, sunflower oil may pose less of a health risk. The estimated dietary in-take values for used edible oil as food ingredients were generally in the order: soya bean oil > mixed vegetable oil > sunflower oil. This implies that people who use soya bean oil are more likely to be exposed to PAHs than people who use other types of oil. A child's daily intake of PAHs from fried finger foods had no effect romf BaP in this study. PAH2, PAH4, and PAH8 daily intakes in children ranged from 0.00 to 3274, 0 to 65.48, and 0 to 65.48 ng kg/bw/day, respectively. Adults who consumed these

finger foods had daily PAH intakes ranging from 0 to 14.6, 0.02 to 0.1, 0.02 to 0.27, 0.02 to 0.60, and 1.20 to 3.14 ng kg 1 bw day 1, respectively for BaP, PAH2, PAH4, and PAH8.

4.11.c Margin of Exposure

The projected margin of exposure (MOE) figures for consumption of used cooking oil as food products and fried finger food can found in Appendix IV. The following is how the MOE value can be interpreted: A MOE value less than 10,000 shows serious negative health consequences whereas a MOE value greater than 10,000 indicates no human side effects (European Food Safety Authority) (EFSA, 2008). After three rounds of frying with the same batch of edible oil, the PAH2, PAH4, and PAH8 MOE figures for adults and PAH8 MOE figures for adolescents consuming finger foods fried with the same batch of edible oil pose no health risk. However, MOE values for PAH2, PAH4 and PAH8 for children consuming finger foods fried with the same batch of edible oil used three times may have health concern since the MOE values were less than 10,000. BaP MOE for the three categories of people has no health effect after three batches of frying in sunflower oil. The MOE values for BaP, PAH2, PAH4, and PAH8 in soya bean oil and mixed vegetable indicated health concerns for the three groups of people. As a result, the MOE and EFSA suggested indicator values for edible oils present health concerns for the three population groups, whereas MOE values for finger foods raise no health concerns for adolescents and adults.

4.11.d Hazards index and incremental lifetime cancer risk

Appendix VI show the non-carcinogenic risk in the form of the hazard index (HI) as a result of human ingestion of used edible oils that form part of food ingredients and finger foods fried in them. The HI values for vegetable oils ranged from 8x 10^{-2} to 2.9 for children, 4 x 10^{-2} to 1.55, and 2.1 10^{-3} to 9.5 10^{-1} for adults, respectively. Some HI figures for children and adolescents consuming oil that has been used in three frying cycles with foods were greater than 1 indicating high non cancer risk for children and adolescents. Nap, Phen and Pyr were the major contributing PAHs congeners. The calculated HI values is higher than those observed by Iwegbue et al (2020) who investigated used oils with fried fish. The HI values for Pyr in soya bean oil, mixed vegetable oil and sunflower oil were 1.5,1.5 and 2.66 respectively for children which is greater than 1. These high HI values for Pyr in some oil samples are concerning because this PAH congener is thought to be carcinogenic in conjunction with BaP. Effiong et al., 2016; Stepanov et al., 2010). Likewise, the HI figure from fried finger foods varied from 1.6 x10^{-2} to 4.45 for children, 3.5 x10^{-2} to 2.35 for adolescents, and 6 x10^{-3} to 1.5 for adults. Nap contributed significant HI values in finger foods which were above the threshold HI value of 1. The high HI values for Nap in finger foods may have high non cancer health concerns to the consumers of such finger foods.

4.11.e Incremental lifetime cancer risk (ILCR) assessment

After three phases of usage as frying oils, the ILCR from these edible oils varied from zero to 2.17 10-2 for children, 0 to 1.104 x 10-2 for adolescents, and 0 to 5.22 10-3 for adults (Appendix VII). After three frying sessions of oils, the cancer risk values for these oil types

were higher than the accepted risk value (1x106) of one in a million chances over a lifetime of 66.3 years. This means that for every 1x106 people who consume these oil types as food ingredients, approximately 21,700 children (1-11 years), 11,021 adolescents (12-17 years), and 5,501 adults (18-70 years) may develop cancer and other non-cancer related health risks. This suggests that consuming these oils has an increased lifetime cancer risk as well as other non-cancer risks. Children are more likely than adolescents and adults to develop cancer. The largest sources to the lifetime cancer risk of the population that consumes finger foods prepared this way in the case of vegetable oil were BaP, I(1,2,3,c,d)P, and B(a)A. Whereas the dominant PAH congeners that contributed ILCR for Iwegbue et al. (2020), who fried fish in edible oils derived from soya bean, olive, palm, sunflower, almond, and mustard seed, were DB(a,h)A and I(1,2,3,c,d)P. The calculated ILCR figures for finger foods fried in these oils are 6.88 x 10-5 for children, 3.67 x10-5 for adolescents, and 1.836 x10-5 for adults, with B(k)F playing a significant role. The indications are that every 1x 10^6 number of people who consumed foods prepared with these oil types, about 68 children, 36 adolescents and 18 adults stand the risk of getting cancer in their life time.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

According to the study's findings, Polycyclic aromatic hydrocarbons were found in both unused as well as used oils. Although LM PAHs predominated over HM PAHs, it was discovered that cyclization and transformation of LM-PAHs to HM-PAHs influenced the levels of HM-PAHs. The concentrations and composition of PAHs increase with the number of frying times, and the effects vary depending on the type of oil used. Sunflower oil has the lowest levels and soya bean oil having the highest levels of PAHs. High proportion of HM-PAHs found in samples increased with increasing number of times the oil is used, that is, third fry register more HM-PAHs than second and first fry. The average percentage detections for LM-PAHs and HM-PAHs were 85.8% and 14.2% respectively whiles that of non-carcinogenic and carcinogenic PAHs were 35% and 65% respectively. The fresh vegetal oils were found to be wholesome and its consumption may not pose any health challenge. Because of the significant levels of PAHs formed during repeated use, particularly the HM-PAHs, which are considered more toxic, soya bean oil was found to be unsuitable for repeated use. The total amount of 4PAH in the used oil types exceeded the European Commission's limit. Benzo(a) Pyrene levels were also higher in some samples than the European Commission's limit. Studies on used edible oils and fried finger foods on human health cancer risk assessment and hazard index (non-cancer) reveal a variety of potential cancer risks. The three age groups studied were found to be at risk for both cancer and non-cancer related health issues,

particularly children. In regards to food safety, edible oil usage should not exceed twice, and if possible, cooking oil reuse should be avoided.

5.2 RECOMMENDATIONS

The following recommendations are made based on the findings of the research and the conclusions reached**:**

1. Polycyclic aromatic hydrocarbons were found to be linked to both carcinogenic and non-carcinogenic risks in all age groups, particularly children, regulatory authorities should conduct increased surveillance on the safety of vegetable edible oils.
2. Repetitive use of edible oil fry food should be avoided. Efforts should be made by governmental bodies responsible as it were to educate the populace more especially the food vendors on the effect of repeated use of oil to fry food. People should be made aware the route of PAHs and harmful effect of consumption of food contaminated with PAHs. Rules and regulations governing PAH levels in the cooking oil in Ghana should be strictly enforced.
3. Edible oil production and packaging should begin with high-quality raw materials and take place in a clean environment to avoid PAHs contaminations in fresh edible oil.
4. There should be education of the public by Ghana Food and Drug Authority for food vendors about how PAHs contamination occur with emphasis on the frying at safely regulated temperatures of edible oil which can curb PAHs contamination.
5. Future research in this area should include cross-frying of food items and should cover the entire country, as regularity excises are relaxed due to logistics constraints.

6. A comprehensive risk assessment of PAH contamination in edible oils should be conducted, and a suitable program to improve the quality and safety of edible oils in Ghana should be established

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APPENDICES

Appendix I

Table 1 Analysis of Variance of PAH Concentrations (µg/Kg) In Soybean Oil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PAHs	15	232698	15513	7.727	0.0041 ***
Residuals	128	256967	2008		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2 Further Test on the specific difference in Means of PAH Concentrations (µg/Kg) in the Soybean Oil

PAHs	mean diff	Lwr	upr	padj
Ant-Nap	93.000	19.171	166.829	0.002
Ant-Acl	91.889	18.060	165.718	0.003
Ant-Ac	154.333	80.505	228.162	0.000
Ant-Flr	138.333	64.505	212.162	0.000
Phe-Ant	-122.556	-196.384	-48.727	0.000
Flu-Ant	-112.889	-186.718	-39.060	0.000
Pyr-Ant	-93.111	-166.940	-19.282	0.002
BaA-Ant	-135.333	-209.162	-61.505	0.000
Chr-Ant	-132.444	-206.273	-58.616	0.000
BaF-Ant	-127.111	-200.940	-53.282	0.000
BkF-Ant	-148.444	-222.273	-74.616	0.000
BaP-Ant	-157.889	-231.718	-84.060	0.000
I123cdP-Ant	-162.000	-235.829	-88.171	0.000
DBahA-Ant	-162.000	-235.829	-88.171	0.000
BghiP-Ant	-162.000	-235.829	-88.171	0.000

Table 3 Analysis of Variance of PAH Concentrations (µg/Kg) In sunflower Oil

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PAHs	15	88948	5930	5.177	0.0001***
Residuals	144	164930	1145		

Table 4Further Test on the specific difference in Means of PAH Concentrations (µg/Kg) in the sunflower Oil

PAHs	mean diff	Lwr	Upr	padj
Ant-Nap	103.333	15.878	190.789	0.006
BaP-Nap	-88.222	-175.678	-0.766	0.046
I123cdP-Nap	-88.222	-175.678	-0.766	0.046
DBahA-Nap	-88.222	-175.678	-0.766	0.046
BghiP-Nap	-88.222	-175.678	-0.766	0.046
Ant-Acl	149.000	61.544	236.456	0.000
Ant-Ac	163.000	75.544	250.456	0.000
Pyr-Ac	90.667	3.211	178.122	0.034
Ant-Flr	163.000	75.544	250.456	0.000
Pyr-Flr	90.667	3.211	178.122	0.034
Phe-Ant	-154.111	-241.567	-66.655	0.000
Flu-Ant	-173.111	-260.567	-85.655	0.000
BaA-Ant	-156.556	-244.011	-69.100	0.000
Chr-Ant	-157.111	-244.567	-69.655	0.000
BaF-Ant	-173.000	-260.456	-85.544	0.000
BkF-Ant	-184.222	-271.678	-96.766	0.000
BaP-Ant	-191.556	-279.011	-104.100	0.000
I123cdP-Ant	-191.556	-279.011	-104.100	0.000
DBahA-Ant	-191.556	-279.011	-104.100	0.000
BghiP-Ant	-191.556	-279.011	-104.100	0.000
Pyr-Flu	100.778	13.322	188.234	0.009
BaF-Pyr	-100.667	-188.122	-13.211	0.009

BkF-Pyr	-111.889	-199.345	-24.433	0.002
BaP-Pyr	-119.222	-206.678	-31.766	0.001
I123cdP-Pyr	-119.222	-206.678	-31.766	0.001
DBahA-Pyr	-119.222	-206.678	-31.766	0.001
BghiP-Pyr	-119.222	-206.678	-31.766	0.001

Table 5 Analysis of Variance of PAH Concentrations (µg/Kg) In Frytol

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PAHs	15	88948	5930	5.177	0.0001 ***
Residuals	144	164930	1145		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 6 Further Test on the specific difference in Means of PAH Concentrations (µg/Kg) in Frytol oil

PAHs	mean diff	Lwr	Upr	padj
Ac-Nap	-54.100	-106.886	-1.314	0.038
BaF-Nap	-53.300	-106.086	-0.514	0.045
BkF-Nap	-63.300	-116.086	-10.514	0.005
BaP-Nap	-61.800	-114.586	-9.014	0.007
I123cdP-Nap	-61.300	-114.086	-8.514	0.008
DBahA-Nap	-63.100	-115.886	-10.314	0.005
BghiP-Nap	-65.400	-118.186	-12.614	0.003
Ant-Ac	66.400	13.614	119.186	0.002
BaA-Ant	-59.500	-112.286	-6.714	0.012
Chr-Ant	-61.800	-114.586	-9.014	0.007
BaF-Ant	-65.600	-118.386	-12.814	0.003
BkF-Ant	-75.600	-128.386	-22.814	0.000
BaP-Ant	-74.100	-126.886	-21.314	0.000
I123cdP-Ant	-73.600	-126.386	-20.814	0.000
DBahA-Ant	-75.400	-128.186	-22.614	0.000
BghiP-Ant	-77.700	-130.486	-24.914	0.000
BghiP-Pyr	-52.900	-105.686	-0.114	0.049

Appendix II

Standard Certificates

676172 Lot: 805414
PAH-Mix 9 1. General
Information

Concentration 10 µg/ml Expanded Uncertainty 5.0 %

Solvent Cyclohexane Expiry Date 01 Oct 2022
Store at 20°C (in the dark) 2.
Composition

Compound	**Conc.[µg/ml]**	**M.W.[g/mol]**	**CAS-No**	**Formula**	
1	Acenaphthene	10.0	154.21	83-32-9	$C_{12}H_{10}$
2	Acenaphthylene	10.0	152.19	208-96-8	$C_{12}H_{8}$
3	Anthracene	10.0	178.23	120-12-7	$C_{14}H_{10}$
4	Benz[a]anthracene	10.0	228.29	56-55-3	$C_{18}H_{12}$
5	Benzo[a]pyrene	10.0	252.31	50-32-8	$C_{20}H_{12}$
6	Benzo[b]fluoranthene	10.0	252.31	205-99-2	$C_{20}H_{12}$
7	Benzo[g,h,i]perylene	10.0	276.33	191-24-2	$C_{22}H_{12}$
8	Benzo[k]fluoranthene	10.0	252.31	207-08-9	$C_{20}H_{12}$
9	Chrysene	10.0	228.29	218-01-9	$C_{18}H_{12}$

10	Dibenz[a,h]anthracene	10.0	278.35	53-70-3	$C_{22}H_{14}$
11	Fluoranthene	10.0	202.25	206-44-0	$C_{16}H_{10}$
12	Fluorene	10.0	166.22	86-73-7	$C_{13}H_{10}$
13	Indeno[1,2,3-c.d]pyrene	10.0	276.33	193-39-5	$C_{22}H_{12}$
14	Naphthalene	10.0	128.17	91-20-3	$C_{10}H_{8}$
15	Phenanthrene	10.0	178.23	85-01-8	$C_{14}H_{10}$
16	Pyrene	10.0	202.25	129-00-0	$C_{16}H_{10}$

Certified on 27 Sep 2021 by Jan Heumann

Instructions for use: Our standards are for laboratory use only and can be used as reference material for calibration of chromatographic systems or related analytical techniques. For handling instructions see the MSDS. Please mix before usage. If particles or precipitation are detected, sonify until solved. The material is homogenous. There is no minimum sample specified. The material in the vial can be used multiple times, but it is strongly recommended, that all external negative influences for the material are considered and ruled out (e.g. high temperatures, UVradiation, moisture, oxygen) and that the weight of the bottle between all uses are noted to remain constant to exclude concentration deviations. It is strongly recommended to open the vial at room temperature only and handle the material under inert gas if necessary. The integrity of the purity cannot be guaranteed, if the substance is handled under unfavorable conditions.

The reported uncertainty U is an expanded uncertainty according to EURACHEM / CITAC guide CG4 – Quantifying Uncertainty in

HPC Standards GmbH | Am Wieseneck 7 | 04451 Cunnersdorf | Germany Phone 0049 34291 337236 | Fax 0049 34291 3372-39 | contact@hpc-standards.com

Certificate of Analysis Page 2/2

Analytical Measurement. The Uncertainty is based on the combined uncertainties, including uncertainties of characterization and stability testing. The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flask. If the substance is proven to be unstable under the given storage conditions, you will be contacted immediately. The warranty of this product is limited to the purchasing price of this product and to the first point of use.

Traceability: The balances used are calibrated with weights traceable to the national standards (DKD).

The HPC Standards GmbH, accredited by DAkkS as indicated by the accreditation number DRM-20844-01-00, has shown competence based on ISO 17034:2017 for production of certified reference materials.

Version	Article	Lot	Reason for Change	Date
2.1	676172	805414	Text update	27 Sep 2021

HPC Standards GmbH | Am Wieseneck 7 | 04451 Cunnersdorf | Germany Phone 0049 34291 337236 | Fax 0049 34291 3372-39 | contact@hpc-standards.com

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LIST OF PAPERS SUBMITTED TO PEER REVIEW JOURNALS AND CONFERENCES PAPERS

1.Suraj. I. S, Gibrilla A. Anita A., Fianko J.R.

Polycyclic Aromatic Hydrocarbons level in finger foods and multiple used edible oils on Ghanaian Market (Under review, Ghana Science Journal)

2. Suraj. I. S, Gibrilla A. Anita A., Fianko J.R.

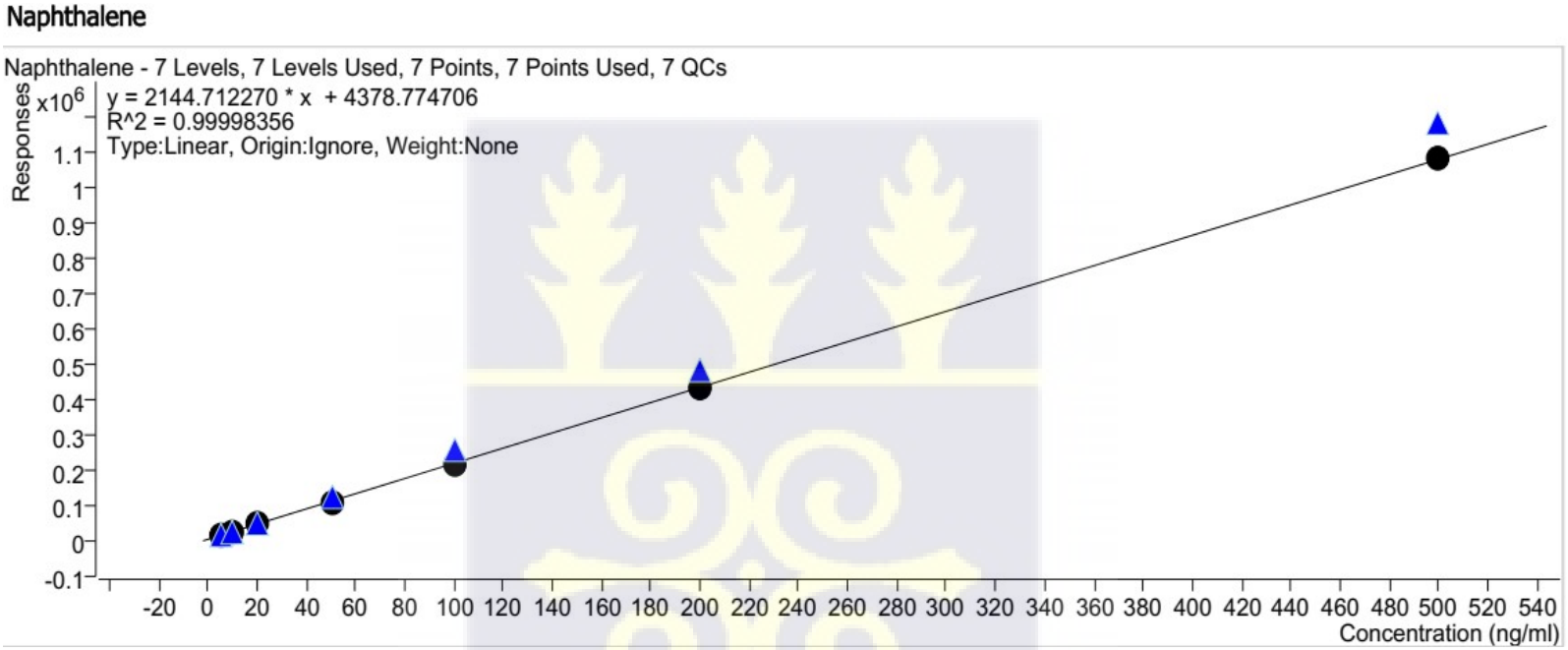
Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Repeatedly Used Edible Oils and Finger Foods on the Ghanaian Market: A Deterministic Risk Analysis (Under review, Journal of Food Science and Technology)

3. One conference paper (Ghana Science Association Conference

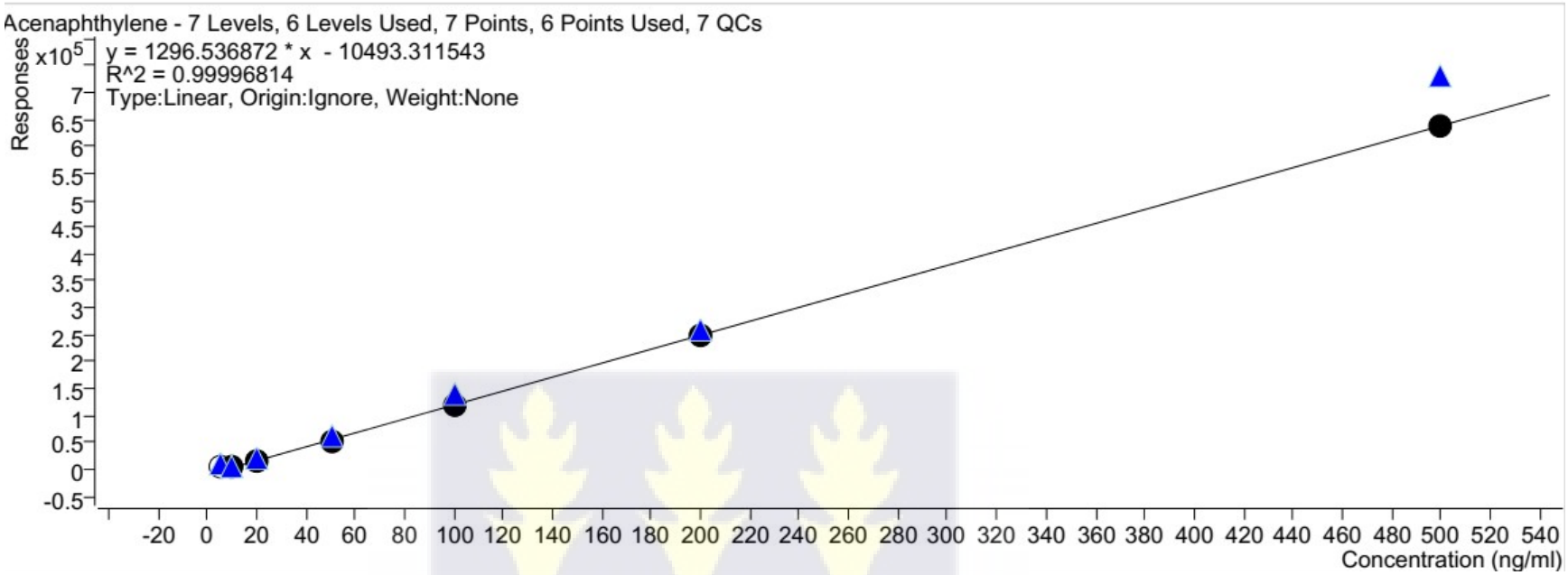
4. Conference abstract acceptance(Conference is in May, 2024 in Austria, Vienna)

Appendix III

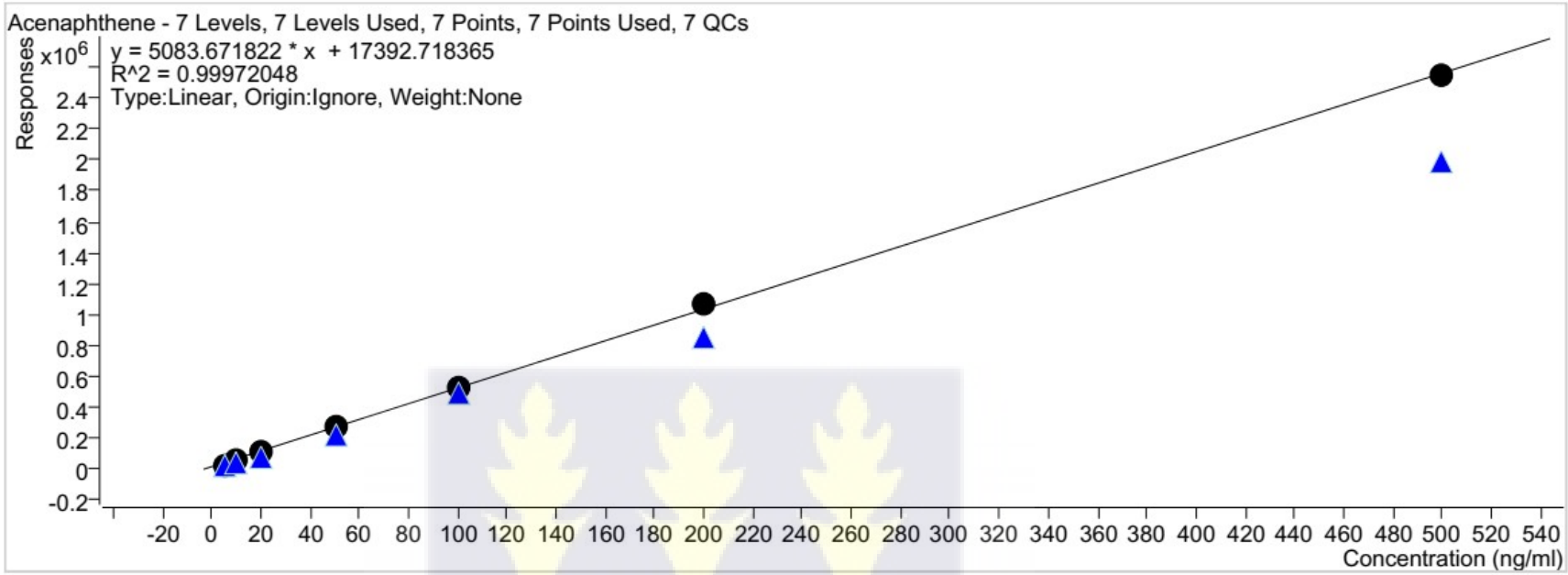
Calibration curves for the quantification of PAHs for the samples



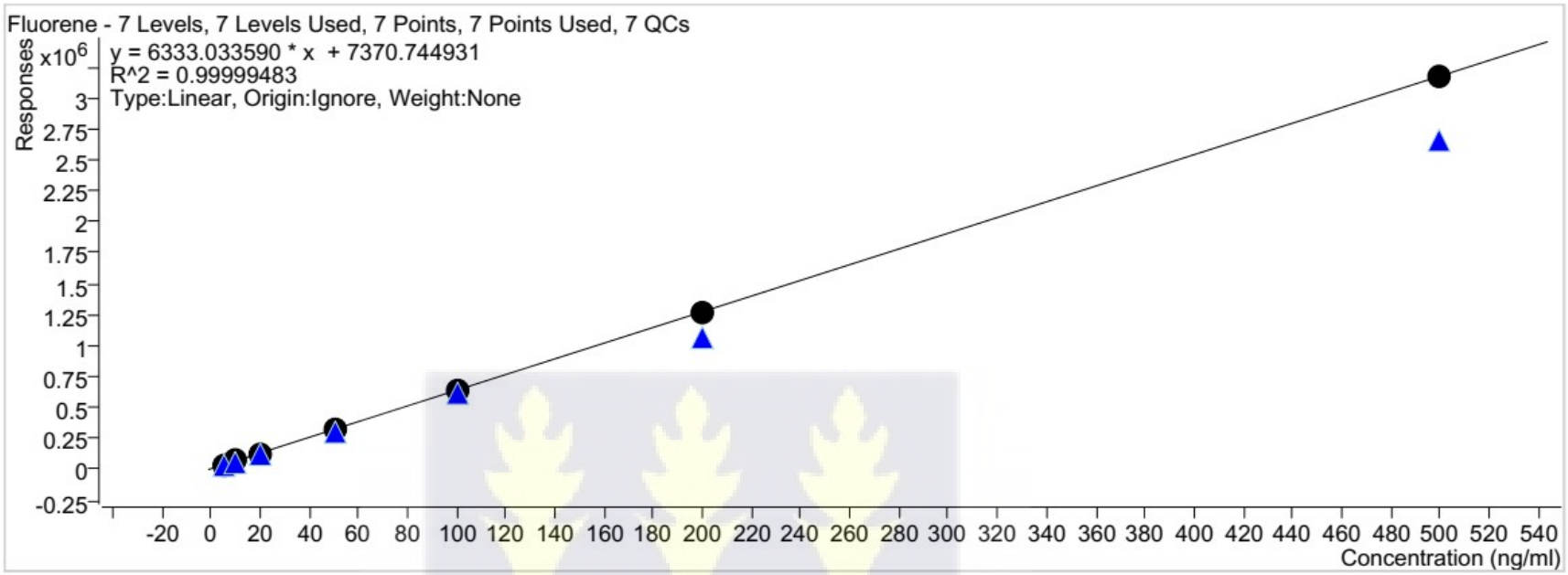
Acenaphthylene



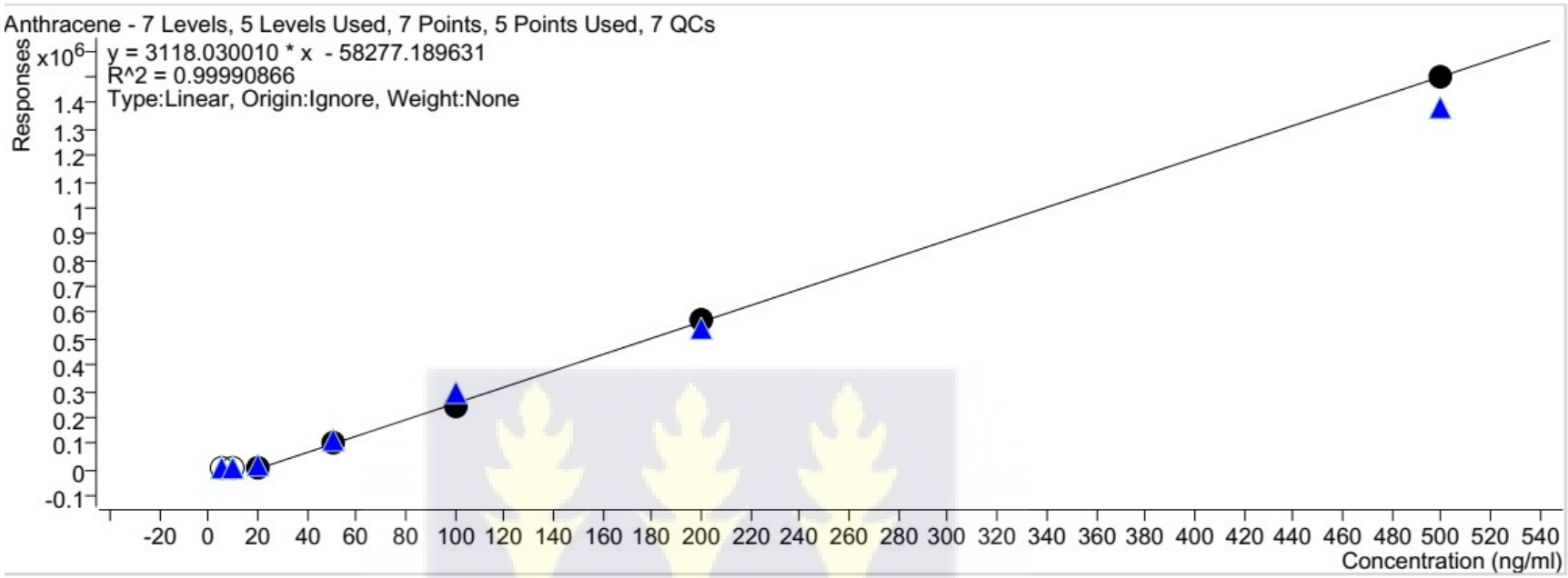
Acenaphthene



Fluorene


Fluorene - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 7 QCs
y = 6333.033590 * x + 7370.744931
R^2 = 0.99999483
Type:Linear, Origin:Ignore, Weight:None
Responses
x10^6
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
-20 0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540
Concentration (ng/ml)

Anthracene


Anthracene - 7 Levels, 5 Levels Used, 7 Points, 5 Points Used, 7 QCs
y = 3118.030010 * x - 58277.189631
R^2 = 0.99990866
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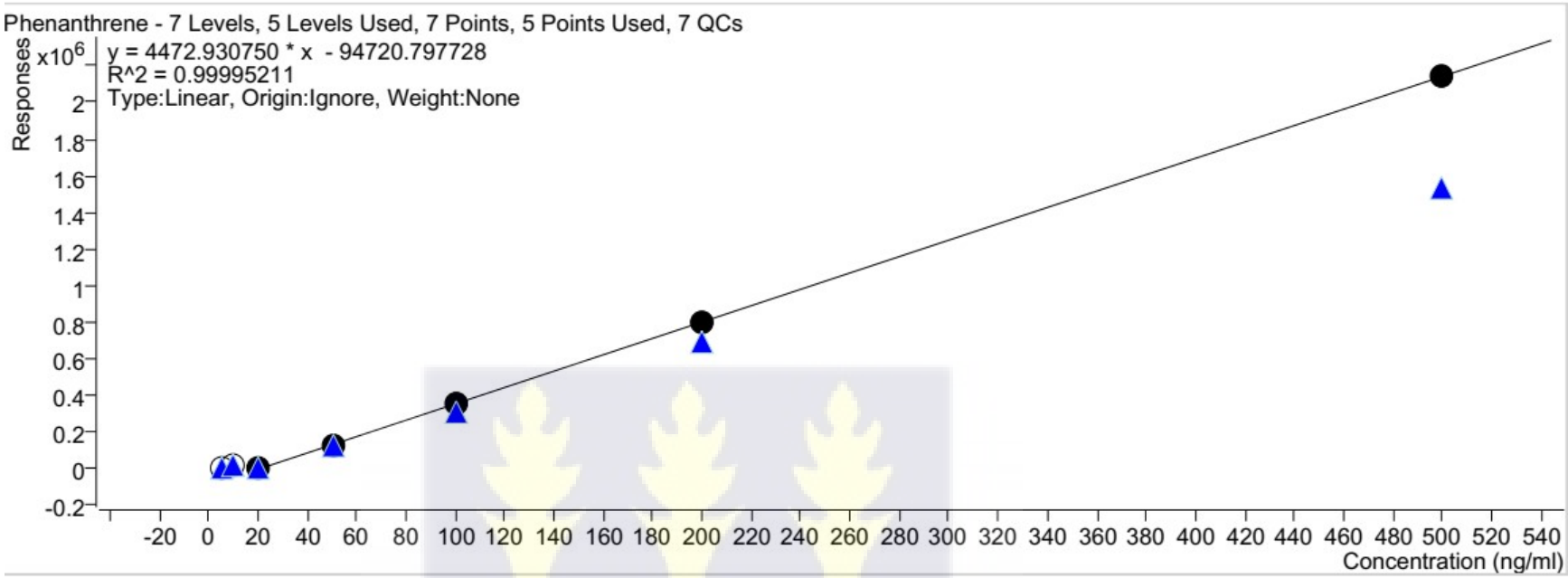
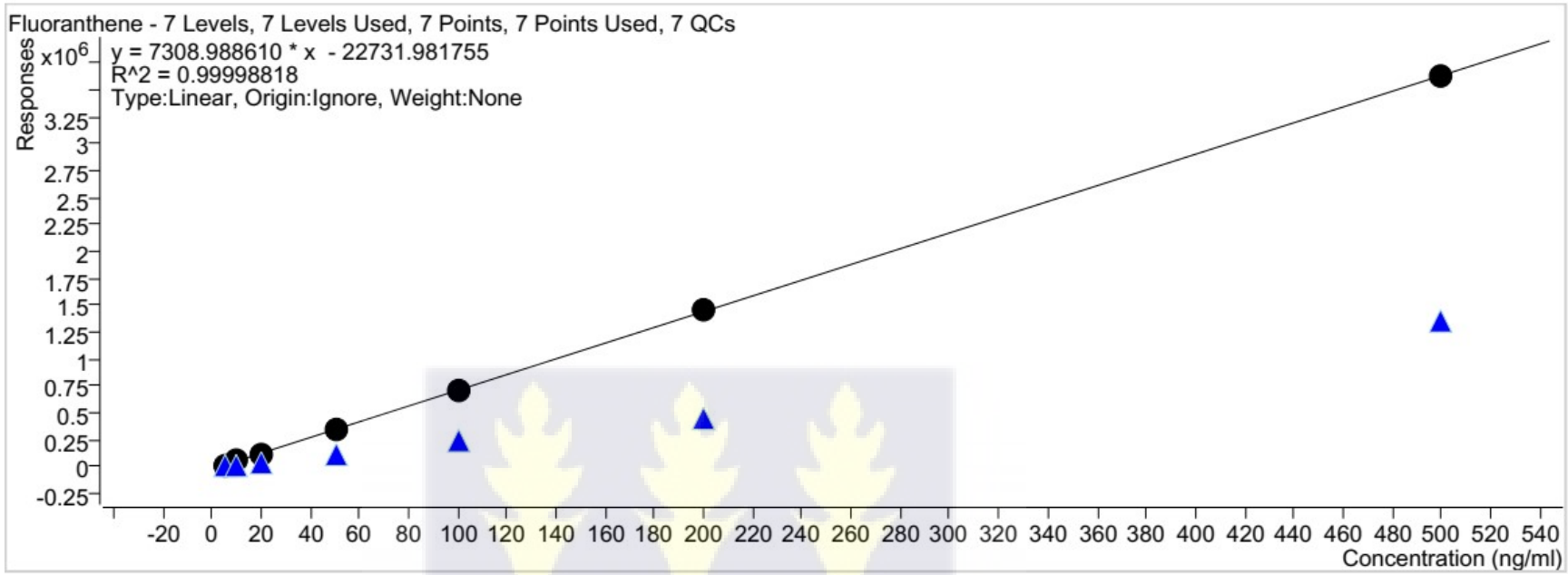
Responses
x10^6
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-20 0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540
Concentration (ng/ml)

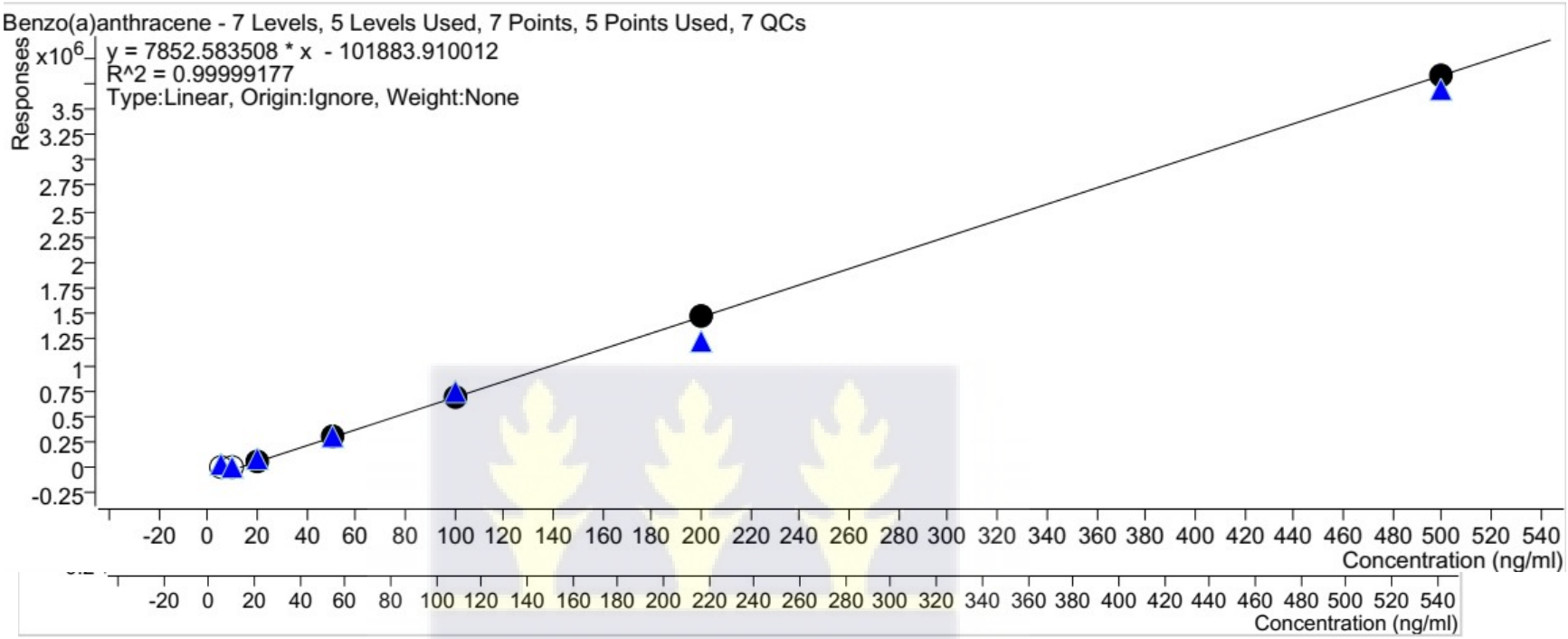
Phenanthrene



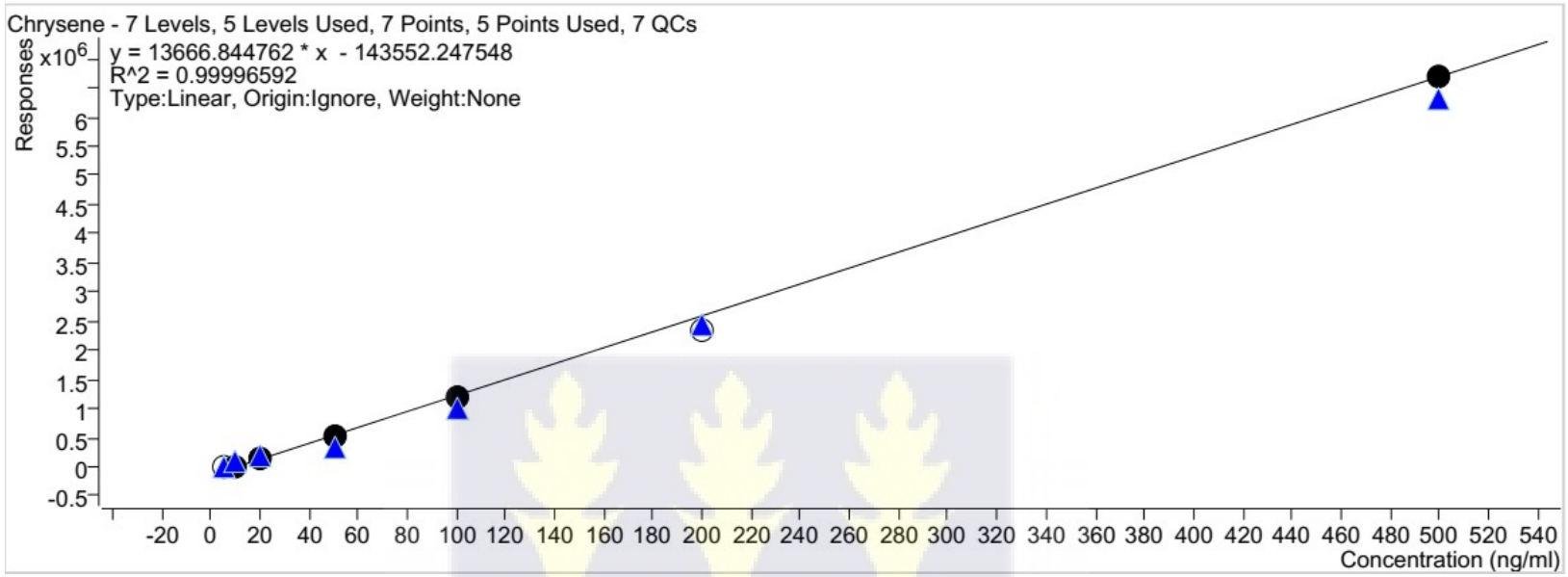
Fluoranthene



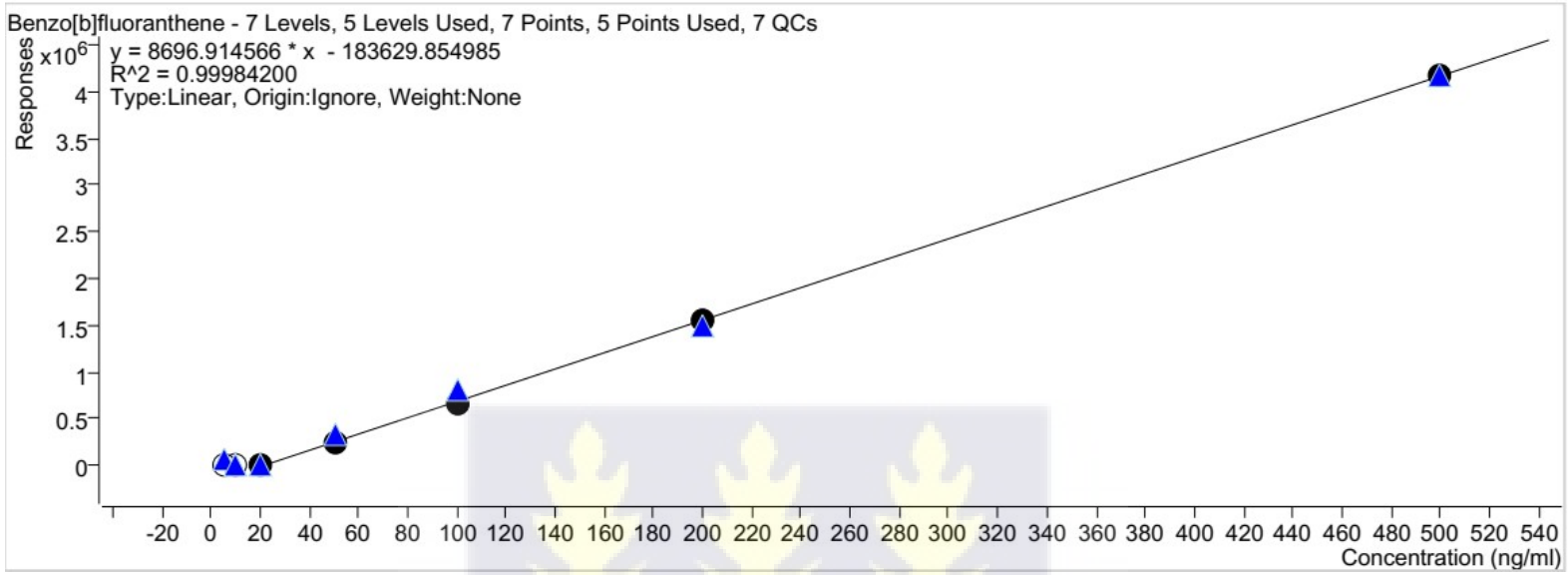
Benzo(a)anthracene



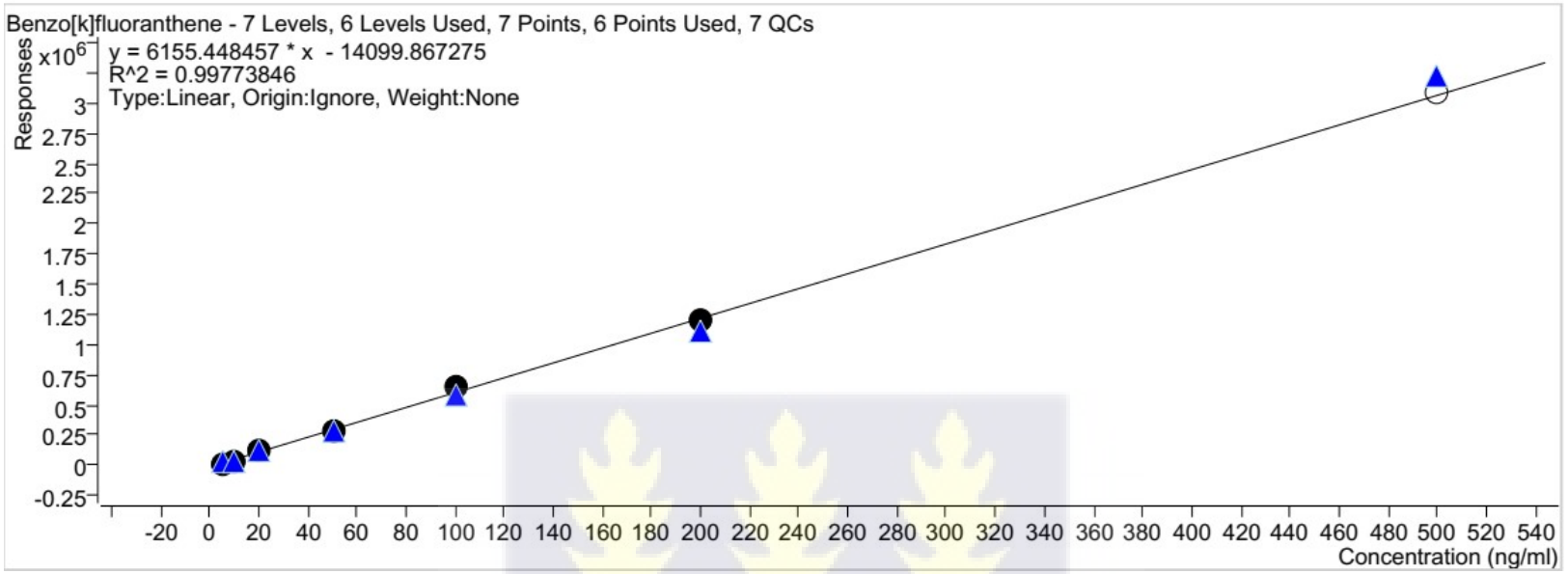
Chrysene


Chrysene - 7 Levels, 5 Levels Used, 7 Points, 5 Points Used, 7 QCs
y = 13666.844762 * x - 143552.247548
R^2 = 0.99996592
Type:Linear, Origin:Ignore, Weight:None
Responses
x10^6
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-20 0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540
Concentration (ng/ml)

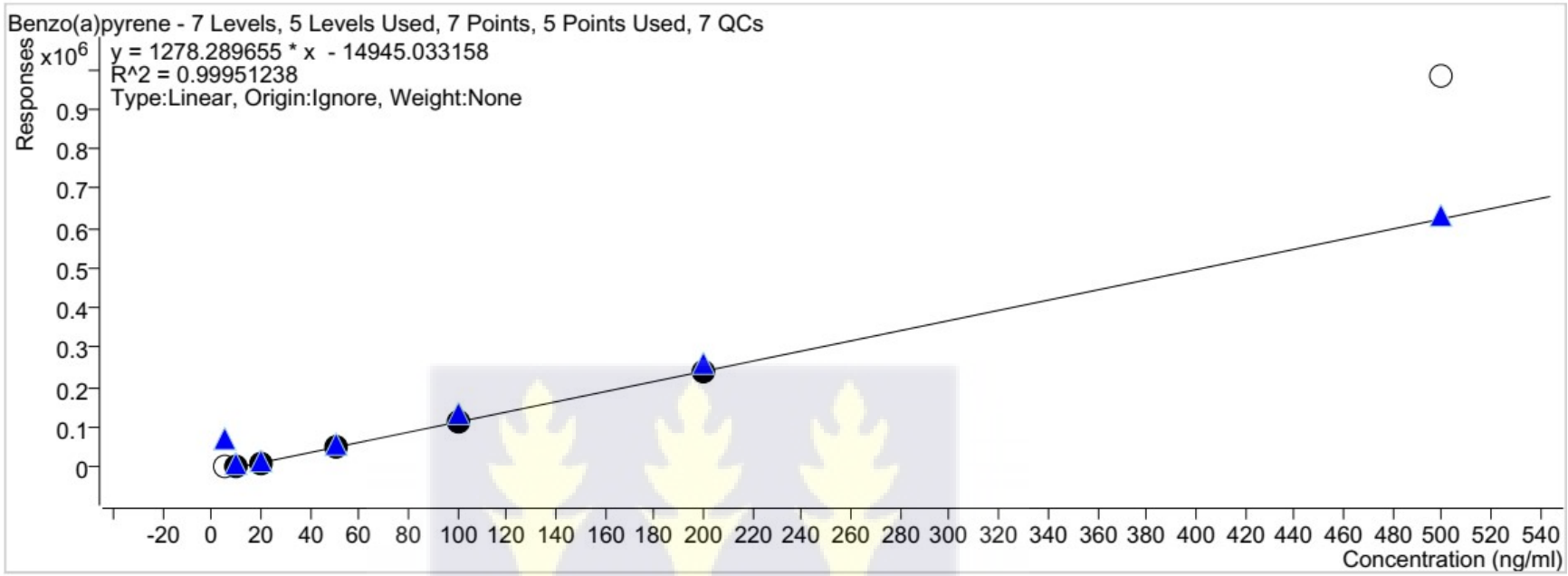
Benzo[b]fluoranthene



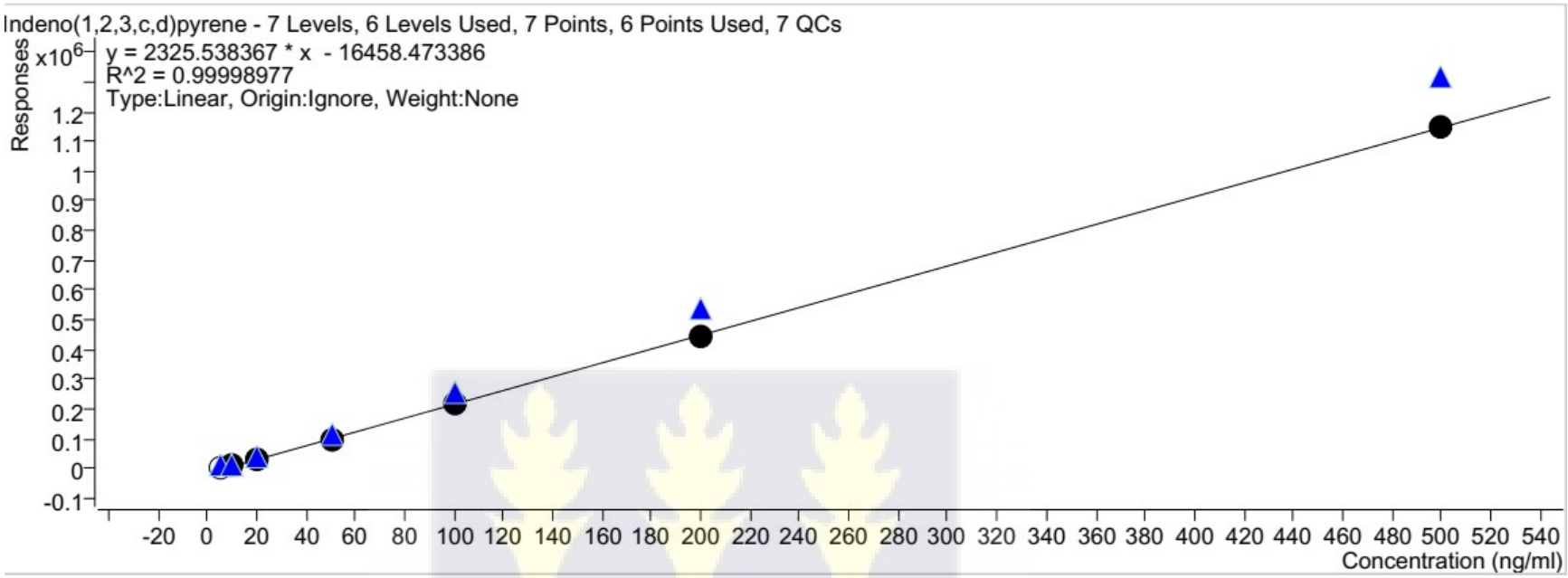
Benzo[k]fluoranthene



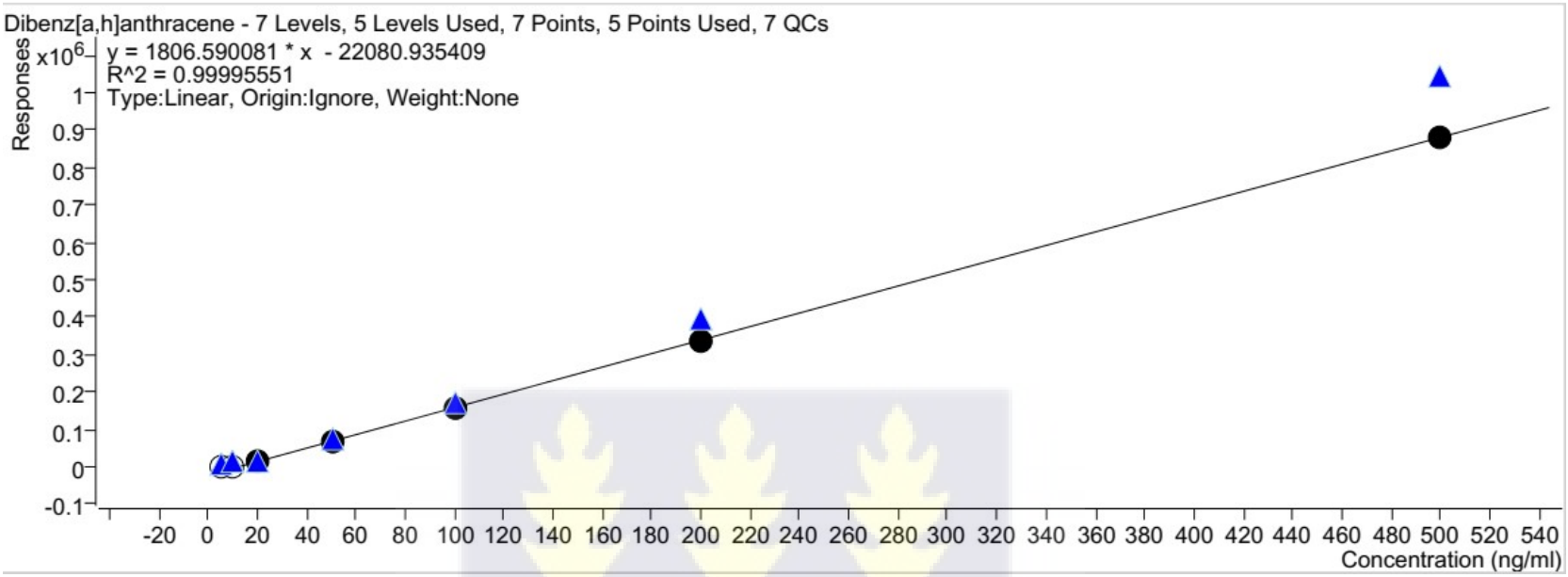
Benzo(a)pyrene



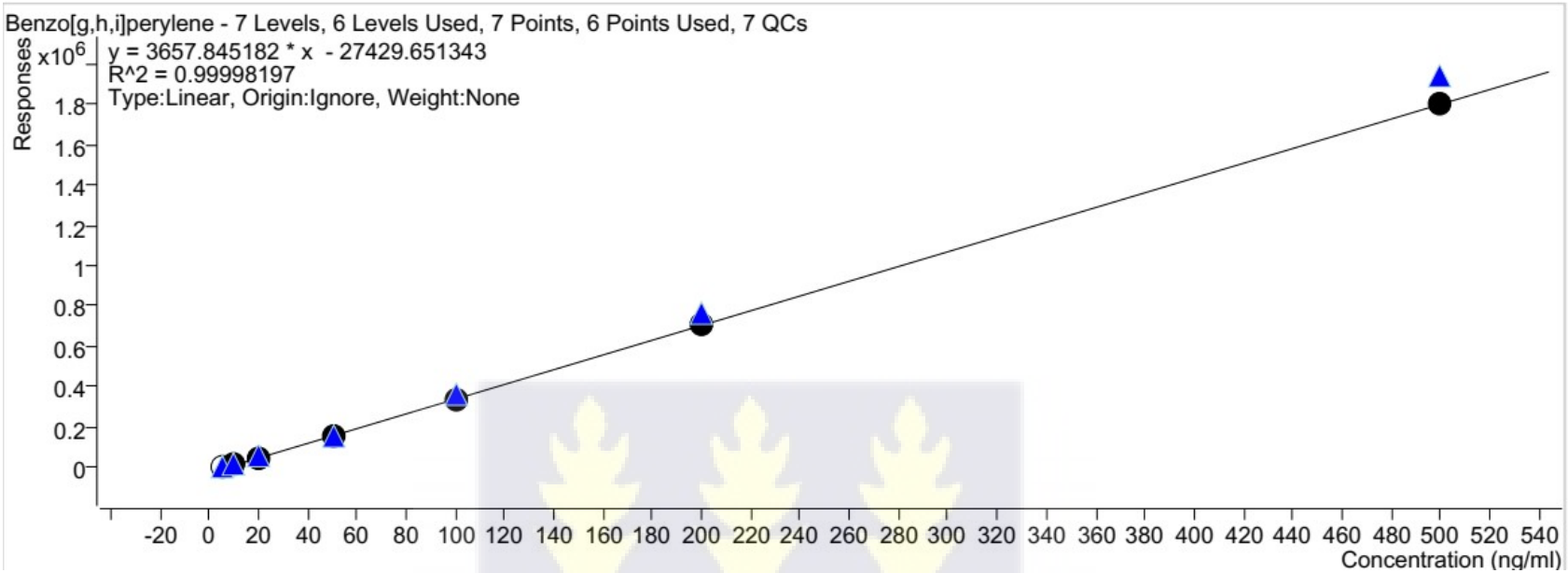
Indeno(1,2,3,c,d)pyrene



Dibenz[a,h]anthracene



Benzo[g,h,i]perylene



Appendix IV

Margin of Exposure for Carcinogenic PAHs

Margin of exposure calculations (MOE)												
	Soya bean oil			mixed oil			Sunflower oil			Finger food		
PAH	children	adolescent	adult	children	adolescent	adult	children	adolescent	adult	children	adolescent	adult
BaP	$1.5x10^3$	$2.8x10^3$	$5x10^3$	$1.4x10^3$	$5.6x10^1$	$1.1x10^2$	0	0	0	0	0	0
2PAH	$4.7x10^2$	$8.4x10^1$	$1.6x10^2$	$1.4x10^3$	2.6x10	5.3x10	$3.8x10^2$	$7.18x10^2$	$1.4x10^3$	$5.31x10^3$	$7.2x10^3$	$11.6x10^4$
4PAH	$3.1x10^2$	$5.86x10^2$	$1.17x10^3$	$5.3x10^2$	$9.9x10^2$	$1.9x10^3$	$3.2x10^2$	$6.09x10^2$	$1.14x10^3$	$5.2x10^3$	$7.3x10^3$	$1.17x10^4$
8PAH	$3.9x10^2$	$7.4x10^2$	$1.48x10^3$	$6.5x10^2$	$1.2x10^3$	$2.4x10^3$	$4.34x10^2$	$8.13x10^2$	$1.63x10^3$	$7.483x10^3$	$10.5x10^3$	$16.9x10^3$

Appendix VI

Calculated Hazard Index values for PAHs

		Hazard Index											
		Soya bean oil			Mixed vegetable oil			Sunflower oil			Finger foods		
PAHs	RfD values	children	adolescent	adult	children	adolescent	adult	children	adolescent	adult	children	Adolescent	Adult
Nap	0.02	2.3	1.2	0.5	2.4	1.25	0.6	2.95	1.55	0.75	4.45	2.35	1.15
Acy	0.06	0.7	0.41	0.206	0.416	0.21	0.111	0.466	0.25	0.1233	0.116	0.066	0.033
Ace	0.06	0.08	0.043	0.166	0.133	0.071	0.035	0.316	0.15	0.081	0.016	0.133	0.006
Flu	0.04	0.37	0.205	0.1	0.475	0.25	0.127	0.475	0.225	0.125	0.0675	0.035	0.017
Phen	0.04	2.7	1.42	0.7	0.75	0.75	0.85	3.25	1.7	0.85	0.225	0.125	0.006
Ant	0.03	0.866	0.456	0.226	1.033	0.533	0.2	0.8	0.433	0.2	0.066	0.046	0.0233
Flt	0.04	0.75	0.435	0.0021	0.825	0.392	0.175	0.3	0.15	0.95	0.175	0.095	0.0475
Pyr	0.03	1.5	0.8	0.4	1.5	0.66	0.7	2.66	1.433	0.7	0.27	0.1433	0.07

Appendix VII

Calculated Incremental Lifetime Cancer risk values

Incremental Life Time Cancer Risk (ILCR) of PAHs													
		Soya bean			**Mixed veg. oil**				**Sunflower oil**		**Finger food**		
PAHs	SFO	Children (10^{-4})	Adolesct (10^{-4}	Adult(10-4	Children (10^{-4}	Adolesct (10^{-4})	Adult(10^{-4})	Children (10^{-4})	Adolesct (10^{-4})	Adult(10^{-4})	Children (10^{-4})	Adolesct (10^{-4})	Adult(10^{-4})
B(a)A	0.73	13.3	6.7	33.9	96.4	51.433	25.7	2.7	89.02	44.5	0	0	
Chr	0.0073	1.4	7.51	3.75	0.842	4.49	2.24	16.69	1.0	5.01	0	0	0
B[b)F	0.73	72.59	38.71	19.38	70.47	3.7588	18.7	8.955	47.7	23.88	0	0	
B[k]F	0.073	6.46	3.447	1.72	1.112	5.93	2.96	3.497	1.87	9.32	6.88	3.67	18.36
B(a)P	7.3	196.0	104.56	0.0052281	20.66	110.21	55.107	0	0	0	0	0	0
I(1,2,3,c,d)P	0.73	0	0	0	20.66	11.58	5.7	0	0	0	0	0	0
DB[a,h]A	7.3	0	0	0	21.7	64.99	32.0	0	0	0	0	0	0

Appendix VIII *

PAHs concentration in (µg/kg) in food items in mixed vegetable oil

Compound	Plantain chips		Doughnut		Beans cake	
Name	Fresh	Fried	Fresh	Fried	Fresh	Fried
Nap	100	776	18	31	78	230
Flr	-	27	-	-	-	10
Ac	-	9	-	-	-	12
Ant	14	68	11	19	1	20
Acl	6	38	5	32	-	20
Phe	-	16	4	12	-	4
Flu	17	45	5	17	-	16
Pyr	20	52	11	19	-	10
B(a)A	8	13	-	-	-	6
Chr	-	20	-	-	-	7
B[b)F	-	-	-	-	-	-
B[k]F	-	-	-	-	-	-
B(a)P	-	-	-	-	-	-
I(1,2,3,c,d)P	-	-	-	-	-	-
DB[a,h]A	-	-	-	-	-	-
B[g,h,i]P	-	-	-	-	-	-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